การสังเคราะห์พอลิแล็กติกแอซิดที่มีปลายโซ่ทั้งสองเป็นประจุบวก

นางสาวนุจรินทร์ ปานสมบัติ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556

SYNTHESIS OF POLY(LACTIC ACID) WITH TWO POSITIVELY CHARGED CHAIN ENDS

Ms. Nutjarin Pansombat

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2013

Thesis Title	SYNTHESIS OF POLY(LACTIC ACID) WITH TWO POSITIVELY CHARGED CHAIN ENDS
By	Ms. Nutjarin Pansombat
Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Assistant Professor Varawut Tangpasuthadol, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……………………………………Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

……………………………………Chairman
(Professor Pattarapan Prasassarakich, Ph.D.)

……………………………………Thesis Advisor
(Assistant Professor Varawut Tangpasuthadol, Ph.D.)

……………………………………Examiner
(Associate Professor Voravee Hoven, Ph.D.)

……………………………………External Examiner
(Assistant Professor Panya Sunintaboon, Ph.D.)

นุจรินทร์ ปานสมบัติ: การสังเคราะห์พอลิแล็กติกแอซิดที่มีปลายโซ่ทั้งสองเป็นประจุบวก. (SYNTHESIS OF POLY(LACTIC ACID) WITH TWO POSITIVELY CHARGED CHAIN ENDS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.วราวุฒิ ตั้งพสุธาดล, 59 หน้า.

งานวิจัยนี้ได้สังเคราะห์พอลิ(แอล-แล็กติกแอซิด) (PLLA)ที่มีปลายโซ่ทั้งสองเป็นประจุบวกจากปฏิกิริยาการเปิดวงอีพอกไซด์ของไกลซิดิลไทรเมทิลแอมโมเนียมคลอไรด์ (GTMAC) ด้วยหมู่ฟังก์ชันที่ปลายโซ่ของพอลิเมอร์ และเพื่อเปรียบเทียบความว่องไวของหมู่คาร์บอกซิลกับหมู่ไฮดรอกซิลในการทำปฏิกิริยากับ GTMAC จึงได้สังเคราะห์ PLLA ที่มีปลายโซ่ทั้งสองเป็นหมู่คาร์บอกซิล (PLLA-diCOOH) หรือหมู่ไฮดรอกซิล (PLLA-diOH) ขึ้นมาก่อน จากนั้นจึงนำไปสังเคราะห์ PLLA ที่มีประจุบวก จากการวิเคราะห์พอลิเมอร์ด้วยเทคนิคโปรตอนเอ็นเอ็มอาร์ พบว่าเปอร์เซ็นต์การแทนที่ของ GTMAC บนปลายโซ่ของ PLLA-diCOOH (สูงถึง 90%) สูงกว่า PLLA-diOH (~19%) ทั้งนี้เชื่อว่ามีสาเหตุมาจากโปรโตเนชันบนวงอีพอกไซด์ด้วยหมู่คาร์บอกซิลที่เป็นหมู่ปลาย ช่วยให้ปฏิกิริยาการแทนที่ของ GTMAC เกิดได้มากขึ้น และจากการวัดค่ามุมสัมผัสระหว่างน้ำกับพื้นผิวของของพอลิเมอร์ที่ได้ขึ้นรูปเป็นฟิล์มเพื่อทดสอบความชอบน้ำของพอลิเมอร์ที่สังเคราะห์ได้นั้น พบว่าค่ามุมสัมผัสบนพื้นผิวของฟิล์มที่เตรียมจากการผสม PLLA ประจุบวก กับ PLLA เกรดทางการค้า สัดส่วน 2:3 โดยน้ำหนัก มีค่าต่ำลงจากค่าของ PLLA เกรดทางการค้าถึง 20% เมื่อทดสอบการแตกสลายด้วยน้ำของพอลิเมอร์โดยแช่ผงพอลิเมอร์ในสารละลายฟอสเฟตบัฟเฟอร์พีเอช 7.4 ที่อุณหภูมิ 37 °C โดยติดตามการลดลงของน้ำหนักโมเลกุลพอลิเมอร์ด้วยเทคนิค GPC พบว่า ที่สัปดาห์ที่ 4 น้ำหนักโมเลกุลของ PLLA ประจุบวกที่สังเคราะห์จาก PLLA-diCOOH (PLLA-COOH+) ลดลง 18% แต่ของ PLLA ประจุบวกที่สังเคราะห์จาก PLLA-diOH (PLLA-OH+) ไม่มีการเปลี่ยนแปลง สมบัติเชิงความร้อนของ PLLA ที่มีประจุบวกทั้งสองแบบ ไม่เปลี่ยนแปลงอย่างมีนัยสำคัญ เมื่อเทียบกับ PLLA-diCOOH และ PLLA-diOH ตามลำดับ

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต..
ปีการศึกษา..................2556.................... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก..........

5472009323: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORDS: POLY(L-LACTIC ACID)/ HYDROPHILICITY/ GLYCIDYLTRIMETHYLAMMONIUM CHLORIDE

NUTJARIN PANSOMBAT: SYNTHESIS OF POLY(LACTIC ACID) WITH TWO POSITIVELY CHARGED CHAIN ENDS. ADVISOR: ASST. PROF. VARAWUT TANGPASUTHADOL, Ph.D., 59 pp.

Poly(L-lactic acid)s having two positively charged chain ends were synthesized by chemical reaction between telechelic PLLA and glycidyltrimethylammonium chloride (GTMAC). In order to compare the reactivity of hydroxyl and carboxyl end groups toward the reaction with GTMAC, two types of telechelic PLLA chains- one with two hydroxyl (PLLA-diOH) and the other with two carboxyl chain ends- (PLLA-diCOOH) were synthesized and used in the reaction. The results obtained by nuclear magnetic resonance (NMR) spectroscopy analysis, revealed that the dicarboxyl end groups of PLLA provided more percentage of GTMAC substitution (up to 90%) than that of the PLLA with hydroxyl end groups (19%). This was because protonation on the epoxide ring by the carboxyl end group helped increase the degree of GTMAC substitution. Changes of polymer hydrophilicity were assessed by means of air-water contact angle measurement of solvent cast films. The contact angle was reduced up to 20% when the positively charged PLLA was blended with a commercial PLLA in a ratio of 2:3 by weight. After immersion of powdered positively charged polymers in phosphate buffer pH 7.4 at 37 ºC, percentage decrease of $\overline{M}_n$ (GPC) at the end of the 4th week of the positively charged PLLA derived from PLLA-COOH (PLLA-COOH+) was 18% while that of the positively charged PLLA derived from PLLA-OH (PLLA-OH+) were insignificantly different. Thermal properties of both two types of the positively charged PLLA were similar to PLLA-diCOOH and PLLA-diOH, respectively.

Field of Study: Petrochemistry and Polymer Science Student's Signature..............
Academic Year: 2013 Advisor's Signature..............

ACKNOWLEDGEMENTS

I would like to express gratitude to my thesis advisors, Assistant Professor Varawut Tangpasuthadol for helpful suggestion, guidance, and encouragement throughout this research. In addition, sincere appreciation is also extended to Professor Pattarapan Prasassarakich, Associate Professor Voravee Hoven and Assistant Professor Panya Sunintaboon for acting as the chairman and examiner of my thesis committee, respectively, and for their valuable constructive comments and suggestions.

I gratefully acknowledge the financial support provided by CU Graduate School Thesis Grant, Chulalongkorn University. Furthermore, I would like to thank all members of Organic Synthesis Research Unit (OSRU), and all my friends, for their friendliness, helpful discussions, cheerful attitude and encouragements during my thesis work. Finally, I also wish to especially thank my family members for their love, kindness and support throughout my entire study.

CONTENTS

Page

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

LIST OF SCHEMES

Scheme Page

LIST OF ABBREVIATIONS

PLA	:	Poly(lactic acid), poly(lactide)
PLLA	:	Poly(L-lactic acid), poly(L-lactide)
ROP	:	Ring-opening polymerization
PC	:	Polycondensation
TNBT	:	Titanium(IV) n-butoxide
$SnCl_2 \cdot 2H_2O$	:	Tin(II) chloride dihydrate
PTSA	:	Para-toluene sulfonic acid
$CDCl_3$	:	Deuterated chloroform
PLLA-diCOOH	:	PLLA with dicarboxyl chain ends
PLLA-diOH	:	PLLA with dihydroxyl chain ends
PLLA-diCOOH+	:	Positively charged PLLA synthesized from PLLA-diCOOH
PLLA-diOH+	:	Positively charged PLLA synthesized from PLLA-diOH
NMR	:	Nuclear magnetic resonance spectroscopy
GPC	:	Gel permeation chromatography
$\overline{M}_n$	:	Number-average molecular weight
$\overline{M}_w$	:	Weight- average molecular weight
PDI	:	Polydispersity index
DS	:	Degree of substitution

CHAPTER I

INTRODUCTION

1.1 Statement of Problem

Poly(*L*-lactic acid), PLLA is an aliphatic polyester derived from *L*-lactic acid (LLA). *L*-lactic acid monomer can be produced by bacterial fermentation of agricultural resources such as corn, cassava and sugarcane [1-3]. This polymer is considered as an environmentally friendly thermoplastic with excellent biodegradability, biocompatibility, transparency, and processibility. Therefore, PLLA has promising applications in biomedical devices, packaging, consumer good, fibers and so on. However, PLLA is rather limited for some applications which directly contact with water such as drug delivery [4, 5] and tissue engineering scaffolds [6-9] due to the lack of hydrophilicity. For using as tissue engineering scaffolds, the hydrophobic PLLA is not appropriate to induce cell adhesion and tissue formation. Hydrophilicity of PLLA can be improved by various techniques, for example, copolymerization with other functional and hydrophilic monomers or blending with other hydrophilic materials [10]. Notwithstanding, copolymerization and blending changes the bulk properties of PLLA which possess negative impact on some applications.

In this work, PLLA with dicarboxyl and dihydroxyl terminated chain ends were firstly synthesized via condensation polymerization. After that, the end groups of those telechelic PLLA chains were subsequently altered from –COOH or –OH to positively charged quaternary ammonium salt. The modification was carried out by chemical reaction between the chain ends of PLLA and glycidyltrimethylammonium chloride (GTMAC), which carries positively charged quaternary ammonium group and an epoxide ring. The reactivity of the chain ends, carboxyl (–COOH) and hydroxyl groups (–OH), were compared as well. Hydrophilicity and hydrolytic degradation of the obtained products were determined by air-water contact angle measurement and immersion of the powdered polymers into phosphate buffer pH 7.4 at 37 ºC for 4 weeks, respectively. Their thermal properties were also investigated by thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC).

1.2 Objectives

The aim of this work was to enhance hydrophilicity of PLLA by chemical modification to provide positively charged units of quaternary ammonium on both chain ends of the polymer. Comparative investigation on of the reactivities of carboxyl and hydroxyl groups toward the epoxide ring opening of GTMAC was also investigated. Finally, hydrophilicity, hydrolytic degradation, and thermal properties of the synthesized polymers were determined.

1.3 Scope of Investigation

1.3.1 Synthesis of telechelic PLLAs, dicarboxyl PLLA (PLLA-diCOOH) and dihydroxyl PLLA (PLLA-diOH) via condensation polymerization from L-lactic acid by using different catalysts e.g. TNBT and a binary catalyst system ($SnCl_2 \cdot 2H_2O$ co-catalyzed by PTSA)

  1.3.1.1 Synthesis of PLLA-diCOOH by adding succinic acid varied from 1 to 4 mol% (compared with mol of L-lactic acid)

  1.3.1.2 Synthesis of PLLA-diOH by using the same condition as the synthesis of PLLA-diCOOH but adding 1,4-butanediol instead of succinic acid

1.3.2 Synthesis of positively charged PLLAs by the reaction between the telechelic PLLAs and GTMAC

  1.3.2.1 Synthesis of PLLA-diCOOH+ derived from PLLA-diCOOH

  1.3.2.2 Synthesis of PLLA-diOH+ derived from PLLA-diOH

1.3.3 Characterization of the telechelic PLLAs and the positively charged PLLAs by using ^1H NMR and GPC

1.3.4 Hydrophilicity test by air-water contact angle measurement of solvent cast films.

1.3.5 Hydrolytic degradation test by immersion of the powdered positively charged polymers into phosphate buffer pH 7.4 at 37 ºC for 4 weeks and following a decrease in molecular weight every week by GPC

1.3.6 Thermal property analysis by TGA and DSC

CHAPTER II
THEORY AND LITERATURE REVIEW

Presently, poly(lactic acid) or polylactide (PLA) is one of the most widely researched materials due to its biodegradability and renewable resources. Furthermore, it has a potential to replace conventional petrochemical-based polymers. In the first part of this chapter, its monomer, and production methods of PLA are presented. In the second part, literature reviews involving chemical and physical modification to improve hydrophilicity of PLA are discussed.

2.1 Lactic acid

Lactic acid (2-hydroxy propionic acid), a bi-functional monomer containing a hydroxyl and a carboxyl group in a molecule, It has been used as a monomer to synthesize PLA. In the first report, it was isolated from milk in early 1780 [3]. This monomer is manufactured by bacterial fermentation or petrochemical process of starch or sugar obtained from natural resources such as corn, sugarcane, and cassava. Because of a chiral center in its molecule, it exists as two optical isomers, L- and D-lactic acid. The L configuration rotates the plane of polarized light clockwise while another rotates that counterclockwise. Lactic acid provided by petrochemical method is an optically inactive, racemic mixture (equimolar mixture of those enantiomers). By the pathway of bacterial fermentation which is more environmentally friendly, this method has been more commercially utilized since the 1990. Lactobacillus, a group of rod-shaped, gram-positive, non-spore-forming bacteria are able to produce lactic acid as a byproduct of glucose metabolism. The fermentation using this type of bacteria results almost 100% in formation of the L isomer (99.5% of the L-form and 0.5% of the D-form) [2].

L-lactic acid D-lactic acid

Figure 2.1 Two optical isomers of lactic acid.

2.2 Poly(lactic acid) and synthesis methods

Poly(lactic acid) (PLA) is a lactic acid based polyester with biodegradability and biocompatibility. Polylactide can also be named in the case of the PLA derived from lactide monomer. This polymer is considered as a chiral polymer due to its asymmetric carbon atoms, two optical isomers (likewise lactic acid and lactide monomer), PLLA and PDLA, appear in PLA (Figure 2.2) [12].

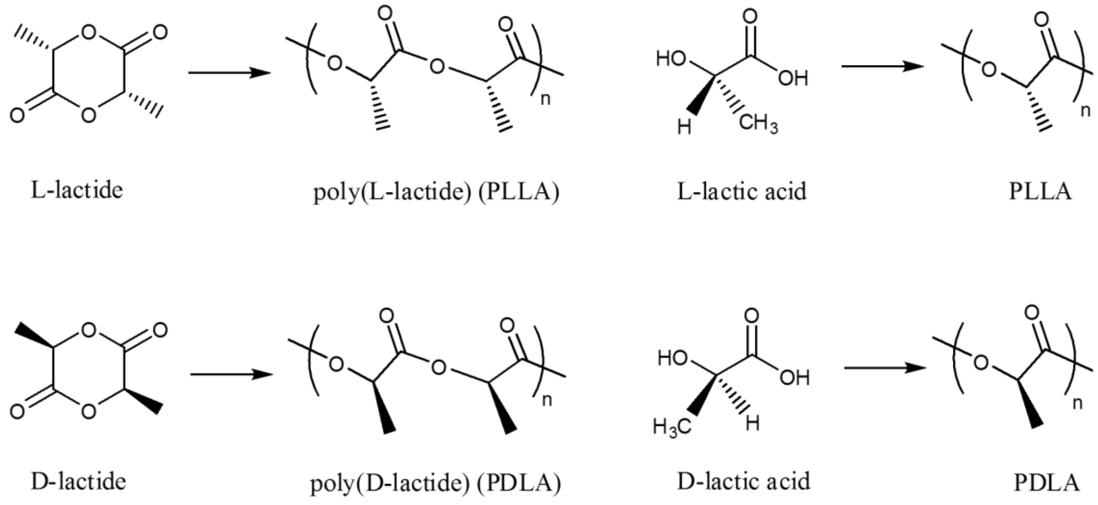


Figure 2.2 Two optical isomers of PLA.

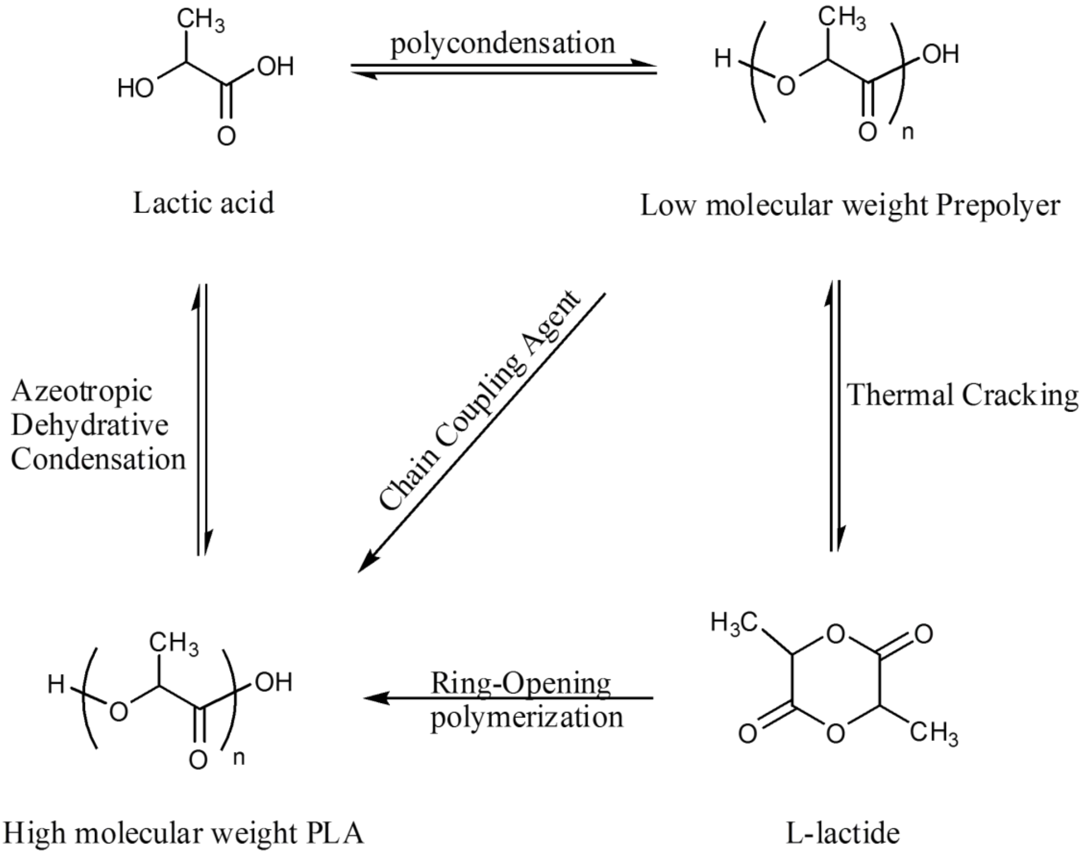
There are two commercial procedures to produce this polymer, ring-opening polymerization (ROP) and polycondensation (PC) (Scheme 2.1) [13, 14].

The ROP method was first introduced by Carothers in 1932 but high molecular weights were not synthesized until in 1954 the high molecular weight PLA was developed by DuPont. Lactide monomer, a cyclic diester is produced by the depolymerization or thermal decomposition of PLA oligomer under reduced pressure. This process can result in high molecular weight PLA (more than 100,000 Da) without water by-product. This pathway is expensive due to complicated purification of lactide.

Lactic acid polymerized via polycondensation yields a low molecular weight polymer but this method is cheaper than ROP. However, chain coupling agent can be utilized to obtain high molecular weight polymer [15-17] including azeotropic dehydrative condensation developed by Mitsui Chemicals which the mixture of lactic acid and a catalyst are dehydrated by refluxing in the presence of high boiling point aprotic solvent such as diphenyl ether under reduced pressure. PLA with M_w more than 3.0×10^5 Da was then produced [13]. Currently, the new process, called melt polycondensation of lactic acid has been commercialized to get significantly high molecular weight PLA. For instance, Moon et al., 2001 have achieved in the synthesis of PLA with M_w of approximately 1.0×10^5 Da via melt/solid polycondensation [18] using Sn (II) catalysts activated by various proton acids. Chen et al., 2006 synthesized PLA by using titanium (IV) n-butoxide (TNBT) as a catalyst without additional reaction promoters. The reaction was accomplished by stepwise extreme decompression of pressure for 7 h, resulted in M_w of PLA reached about 130,000 Da [19]. In 2011, Song and Wu synthesized PLLA with M_w up to ~50,000 Da via melt/solid poly condensation in a short polymerization time (4 h) by adding a protonic acid, para-toluene sulfonic acid (TSA) at the dehydration step and $SnCl_2$ at the melt polymerization step with appropriate temperature and pressure [20].

Polycondensation of lactic acid in the presence of difunctional monomers such as dicarboxylic acids and diols can also be conducted to obtain telechelic PLA, dicarboxyl-terminated and dihydroxyl-terminated PLA as reported in many literatures. For example, in 1996 Hiltunen and co-workers synthesized lactic acid based telechelic prepolymers by using 1,4-butanediol and adipic acid to prepare those polymers [21]. Zeng *et al.*, 2009 synthesized dihydroxyl terminated PLLA by adding a small amount of 1,4-butanediol (1.5 mol%) via condensation polymerization in order to prepare poly(ester urethane) via chain-extension reaction in the presence of 1,6-

hexamethlyene diisocyanate (HDI) as a chain extender [22]. Inkinen *et al.*, 2011 reported the synthesis of telechelic PLLA prepolymers by using 1,4-butanediol, pentaerythritol, adipic acid, or 1,2,3,4-butanetetracarboxylic acid as co-monomers, they also revealed that longer polymerization time could reduce amount of free PLLA chains not attached to those co-monomers investigated by ^{1}H NMR analysis [23].



Scheme 2.1 Synthesis methods of PLA.

In this work, dicarboxyl-terminated and dihydroxyl-terminated PLLA were synthesized through the PC method by adding a small amount of succinic acid and 1,4-butanediol, respectively. The synthesis method was followed the researches of Chen *et al.* [19], Zeng *et al.* [22], and Song *et al* [20]. The obtained PLLA with molecular weight in the range of 3,000 to 9,000 Da could be varied as a function of succinic acid or 1,4-butanediol. TNBT and $SnCl_2$/TSA were used as catalysts to synthesize the polymers.

A possible mechanism of polycondensation in the presense of TSA and Tin(II) catalyst, an effective system proposed by Moon et al. [18] is shown below:

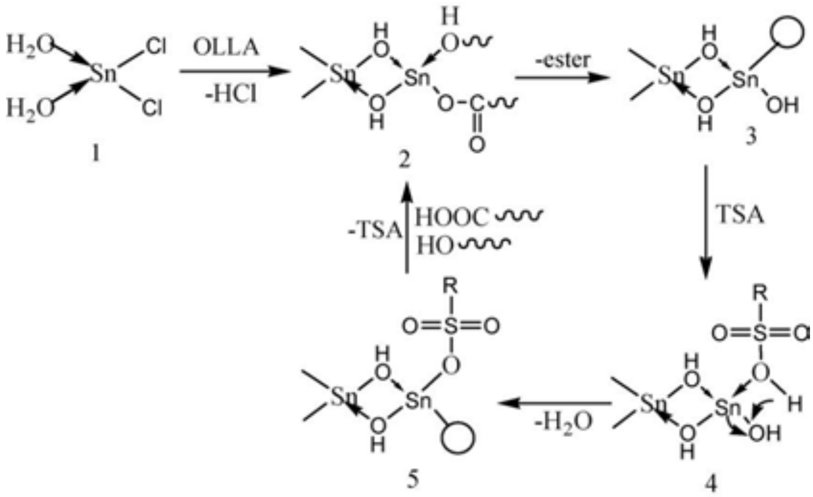


Figure 2.3 The proposed mechanism of polycondensation in the presence of TSA and Sn(II) catalyst.

The mechanism starts with the end groups of PLLA forming coordinate bonds with Sn(II) (2). When the molecular weight of the PLLA is high enough, terminal hydroxyl and carboxyl groups would decrease resulting in the coordination site vacancy of the catalyst (3). TSA added acts as a ligand to fill the vacancy to prevent site reaction (4). Addition of a strong proton acid like TSA also stimulates the dehydration of compound 3 to 5 so that the rate of reaction becomes higher. Moreover, TSA can also reduce product discoloration caused by many factors e.g. high reaction temperatures, long polymerization time, catalyst used, solvents and byproducts during the reaction [1].

2.3 Hydrophilicity improvement in PLA

Aliphatic polyesters including PLA are classified as biodegradable polymers and have been frequently utilized in biomedical products such as sutures, bone screws, tissue engineering scaffolds and drug delivery systems. Although they have been introduced for several years, improvements of their bulk or surface propertied are still required. The hydrophobic PLA is rather limited for using in some biomedical field as mentioned earlier. In order to increase hydrophilicity of PLA, various techniques, for example, copolymerization with other functional and hydrophilic monomers or blending with other hydrophilic materials have been reported.

In 2003, Wan and colleagues [9] emphasized that hydrophilicity improvement for PLLA is necessary in order to prepare tissue engineering scaffolds. Because the polymer is quite hydrophobic, penetration of cells into the pores of the scaffolds is hindered. Nutrient delivery and waste removal are also inhibited. They modified the bulk properties of PLLA to enhance its hydrophilicity using poly(ethylene glycol) (PEG). PEG is well-known polymer, which is extensively utilized to improve the hydrophilicity of several hydrophobic materials. Diblock and triblock copolymers of PLLA/PEG (PLE) were synthesized by ring opening polymerization of L-lactide in the presence of PEG and appropriate catalysts. By using succinic anhydride as a coupling agent, multiblock PLE copolymers were prepared as well. Although hydrophilicity of the copolymers was enhanced but the cell attachment on the multiblock PLE surface was not better than that on unmodified PLLA film. They explained that PEG was not good enough for cell affinity.

A copolymer, poly(L-lactide)-g-poly(N-vinyl pyrrolidone) (PLLA-g-PVP) synthesized by γ-ray irradiation of PLLA and N-vinyl pyrrolidone was presented in the literature of Chen et al. in 2009 [24]. The obtained copolymer not only showed greater hydrophilicity but also higher degradation rate compared with PLLA.

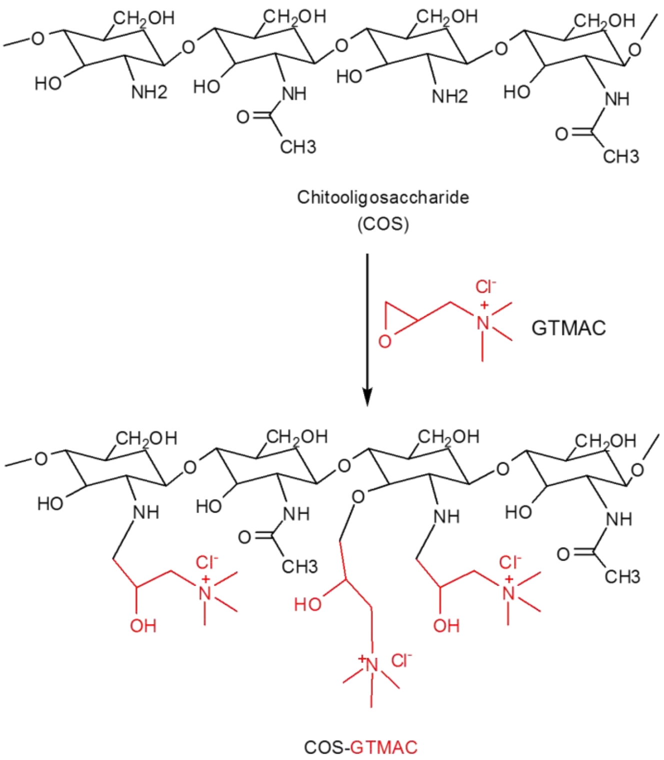
By blending PLA with natural biodegradable dextran, a sponge-like scaffold was prepared to improve the cell affinity on the scaffold by dissolving them in organic solvent as reported by Cai et al. in 2002 [6]. The hydrophilicity of the prepared polymer was significantly enhanced including cell adhesion and cells proliferation on the scaffold were also better than unmodified PLA. However, this blending polymer lowered mechanical properties both tensile strength and elongation due to the incompatibility between hydrophobic PLA and hydrophilic dextran.

Wan et al., 2006 [8] blended PLLA with chitosan, which has amino and hydroxyl groups on its backbone. It was found that blending chitosan with PLLA was very difficult at a highly miscible level. After blending, contact angle of the polymer surface was lower, this means that hydrophilicity was improved. Nevertheless, decrease in polymer strength and thermal stability became unavoidable problems.

2.4 Glycidyltrimethylammonium chloride (GTMAC)

GTMAC is a compound consisting of a reactive epoxide and a positively charged of quaternary ammonium group. It's not only readily soluble in water but also exhibit anti-bacterial activity. Its structure contains a reactive epoxide, which is ready to react with a nucleophile. It was broadly used for preparation of positively charged polymer.

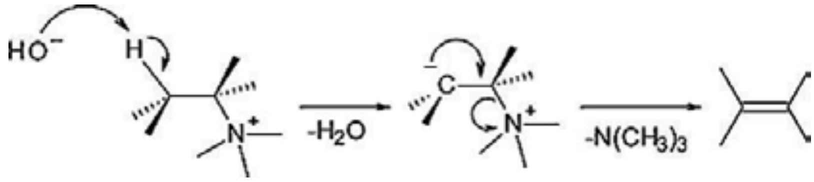
In 2003, Kim *et al*. synthesized a functionalized chitooligosaccharide (COS-GTMAC) by reaction of COS with GTMAC [25]. The reaction involves nucleophilic substitution and cleavage of the epoxide ring. An amino group of chitosan acted as a main nucleophile while hydroxyl group could also react with epoxide ring under neutral and alkaline condition (Scheme 2.2). They found that the degree of substitution (DS) is dependent on conditions such as pH, mole ratio, temperature and time. The DS gradually increased with increasing reaction time and after 24 h, it reached ~115%. The higher DS value than 100% illustrates that the epoxide of GTMAC can react with hydroxyl group as well. Notwithstanding, the reactivity of an amino group is still much higher than that of a hydroxyl group at neutral condition. The obtained COS-GTMAC displayed higher antimicrobial activity and more readily dissolve in water than the unmodified COS.



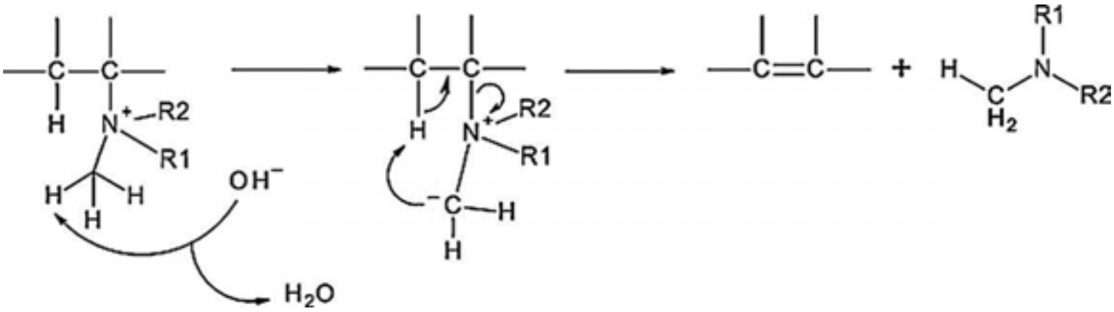
Scheme 2.2 Reaction of chitooligosaccaride with GTMAC.

In 2011, cationic poly(vinyl alcohol) was synthesized by Fatehi et al. [26] with varied conditions such as ratios of GTMAC to PVA, temperature, time, and solvent. The efficiency of the cationic modification was rather low because the hydrolysis of both GTMAC and cationic-modified PVA occurred and more hydrolysis of GTMAC took place under the alkaline conditions. It resulted in formation of N-(2,3-dihydroxy)propyl-N,N,N-trimethylammonium chloride, and dimer and trimer of GTMAC.

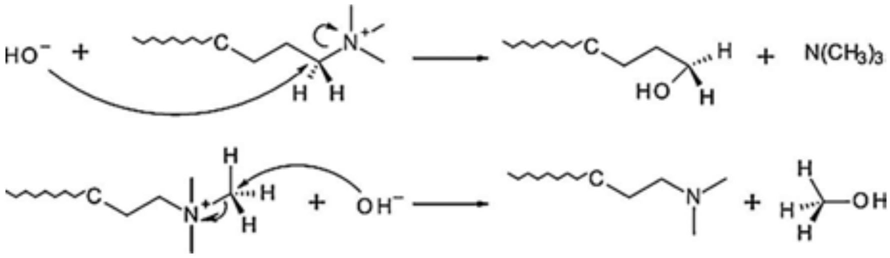
Additionally, for degradation of quaternary ammonium group in alkali condition, various mechanism pathways were proposed [27]. By E_2 elimination reaction called "Hofmann elimination", the quaternary ammonium group can be cleaved to yield an amine and an alkene. This is caused by a substituent in the β-position of the nitrogen atom contains at least one hydrogen atom (Scheme 2.3). When the molecule bearing the ammonium group consists of α and β carbon atoms which have steric hindrance, Ei elimination reaction also occurs. Hence, hydroxide ion can react with a proton of one methyl group on the ammonium to form an alkene and an amine (Scheme 2.4). As illustrated in Scheme 2.5, a carbon atom in α-position of the ammonium group is attacked by a hydroxide ion via S_N2 reaction, results in formation of an alcohol and an amine.



Scheme 2.3 Degradation of quaternary ammonium by Hoffmann elimination.

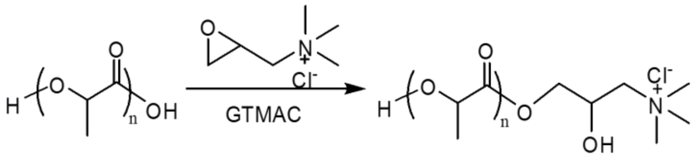


Scheme 2.4 Degradation of quaternary ammonium by Ei elimination.



Scheme 2.5 Degradation of quaternary ammonium via S_N2.

In the previous study, Piyachai Khomein, a member of Tangpasuthadol group used GTMAC to react with PLLA. He reported in his Master’s thesis that carboxyl (–COOH) chain end mainly reacted with GTMAC and hydrophilicity of the polymer was improved as determined by contact angle measurement of molten polymer (Scheme 2.6).



PLLA p-PLLA

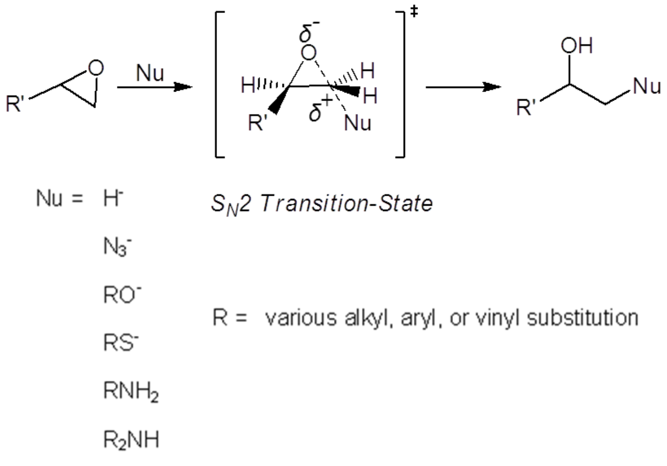
Scheme 2.6 Reaction of PLLA with GTMAC.

From the mentioned studies, GTMAC displays as an effective compound, which can be evidently used to synthesize positively charged polymers and exhibits hydrophilicity. Therefore, this work chose GTMAC for producing the positively charged PLLA.

2.5 Epoxide Ring Opening

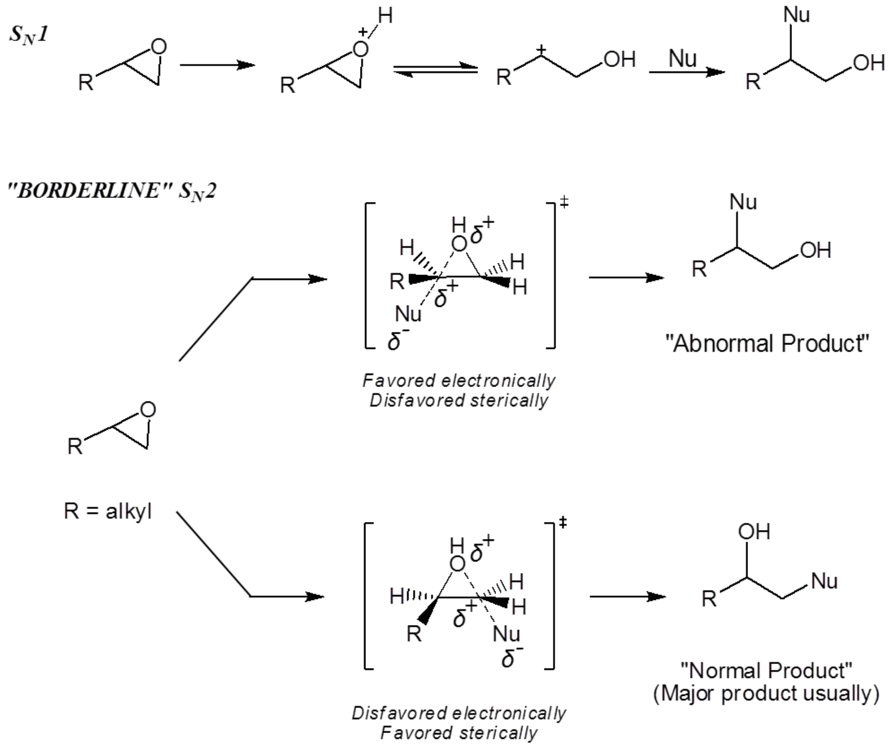
Ring opening reaction of epoxide involves nucleophilic substitutions, S_N1 and S_N2. Type of the reactions favoured depends on pH.

In neutral or basic conditions including a strong nucleophile, S_N2 mechanism is dominant (Scheme 2.7). Due to ring strain, bond breaking is more preferred than bond forming in the transition-state relative to a normal S_N2 reaction. In the transition-state the carbon atom at which replacement occurs bears a partially positive charge. Normal S_N2 takes place to perform nucleophilic attack at the least substituted carbon (least steric hindrance) with inversion of stereochemistry.



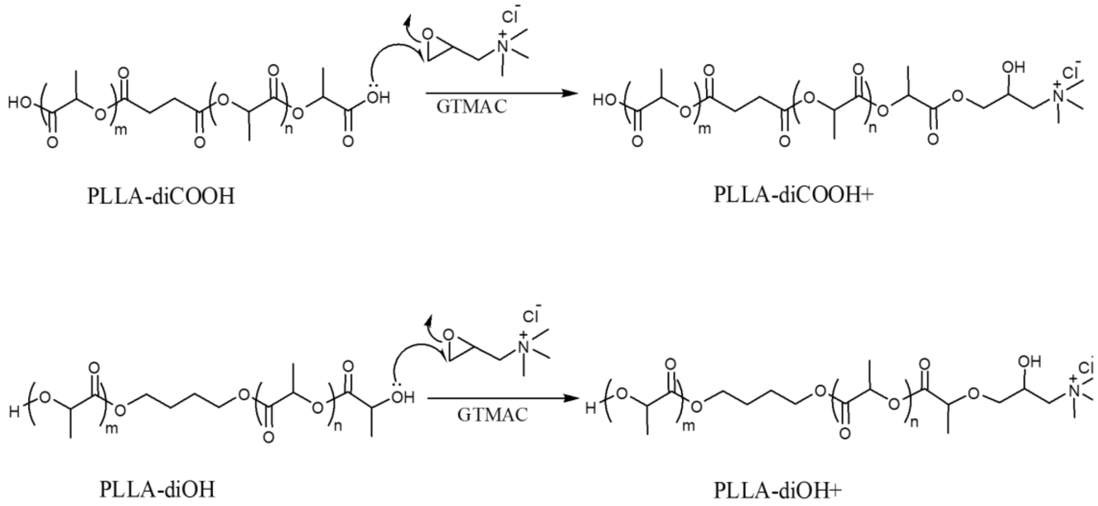
Scheme 2.7 Ring opening mechanism of epoxide in neutral of basic condition.

In acidic condition and a poor nucleophile, both S_N2 and S_N1 are possible to take place. Because of acidity, the epoxide will be protonated first, the ring cleavages, followed by nucleophilic attack on carbocation center. This mechanism is classified as S_N1 that steric factors are important less than observed in S_N2 reactions. If the conjugate acid of the epoxide formed after protonation is then attacked by nucleophile causing the ring is opened, this is S_N2. Consequently, the reaction of epoxide in acidic condition gives mixtures of the two nucleophilic substitution reactions.



Scheme 2.8 Ring opening mechanism of epoxide in acidic condition.

In this work, the reaction of GTMAC with PLLA-diCOOH and PLLA-diOH were carried out in neutral and basic conditions, therefore S_N2 mechanism should be favored as illustrated in Scheme 2.6. Polar aprotic solvents, DMSO (polarity index = 7.2) and DMF (polarity index = 6.4), were chosen for this work because these solvents are especially useful in S_N2 reaction, vastly increase reaction rate and can dissolve both GTMAC and the synthesized polymers. Polar aprotic solvent cannot form hydrogen bonds and do not solvate anions to any appreciable extent. Thus, anions are unencumbered by a layer of solvent. These "naked" anions are very reactive both as bases and nucleophiles. And we also compared the efficiency of DMSO (polarity index = 7.2) and DMF (polarity index = 6.4) having different polarity by the term of degree of substitution.



Scheme 2.9 Ring opening mechanism of GTMAC with PLLA-diCOOH and PLLA-diOH.

2.6 Hydrolytic Degradation

The principal mode of degradation for lactic acid-based polymers is hydrolysis of main chain ester bonds and the rate and extent of degradation depends on the character of polymer (e.g. hydrophilicity and crystallinity) [1].

After hydrophilicity of PLLA was improved, we expected that the rate of hydrolytic degradation would also increase because if the polymer chain is more hydrophilic, water molecules can more easily diffuse into the polymer chains. This is to follow whether or not the chains are broken down into smaller chains. Eventually, it becomes lactic acid. The hydrolysis mechanism of PLA is shown in Figure 2.3.

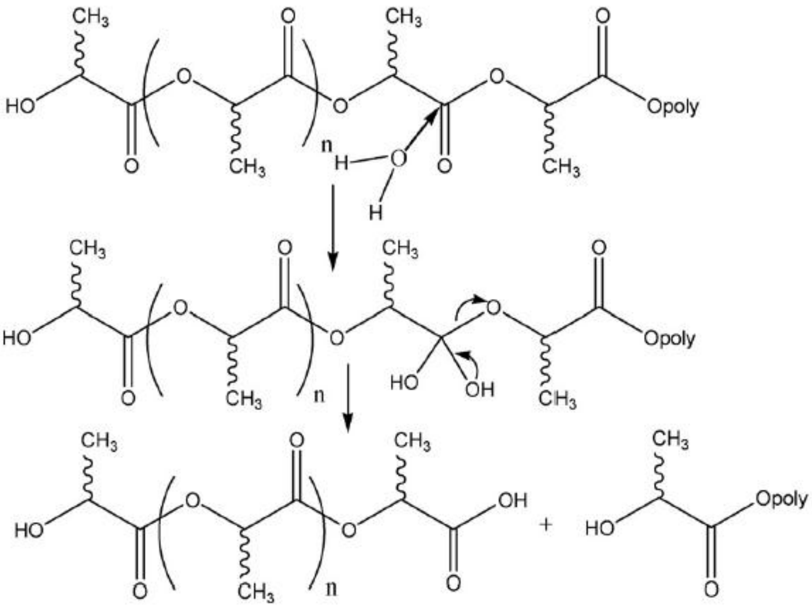


Figure 2.4 Hydrolysis of PLA [1].

In summary, this work emphasized on the alteration of PLLA chain ends from –COOH or –OH to positively charged quaternary ammonium salt to enhance hydrophilicity of the polymer, which could also increase the rate of hydrolytic degradation.

CHAPTER III

EXPERIMENTAL

3.1 Materials

L-lactic acid solution (LLA, 88 wt%) was purchased from Carlo Erba Reagent (France). Titanium (IV) n-butoxide (TNBT, reagent grade 97%), tin(II) chloride dihydrate ($SnCl_2•2H_2O$), triethylamine (NEt_3), 1,4-butanediol, para-toluene sulfonic acid (PTSA), succinic acid, and glycidyltrimethylammonium chloride (GTMAC) were purchased from Sigma-Aldrich (USA). Methanol (MeOH), ethanol (EtOH), sodium chloride (NaCl), chloroform-d were purchased from Merck (Germany). Chloroform, tetrahydrofuran (THF) (HPLC grade) dimethyl formamide (DMF), and dimethyl sulfoxide (DMSO) were purchased from RCI Labscan Limited (Thailand). All chemicals were used as received, except GTMAC that was dehydrated by high vacuum pump before use.

3.2 Equipments

3.2.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

^1H NMR spectra of the samples dissolved in $CDCl_3$ were recorded on a Varian, model Mercury-400 nuclear magnetic resonance spectrometer (USA) operating at 400 MHz and a Bruker Advance-III 300 operating at 300 MHz at room tempertaure, Chemical shifts were reported in part per million (ppm) relative to tetramethylsilane (TMS) or using the residual protonated solvent signal as a reference.

3.2.2 Gel Permeation Chromatography (GPC)

Molecular weight of the synthesized polymers were obtained from Waters 600 controller chromatograph equipped with two HR (Waters) columns (HR1 and HR4) (MW resolving range = 100-500,000 Da) at 35 °C and a refractive index detector (Waters 2414). THF (HPLC grade) was used as a solvent for polymers and an eluent for GPC with a flow rate of 1.0 mL/min. Sample injection volume was 50 μL. The molecular weight of polymers was reported in Dalton unit relative to polystyrene standard (Seven polystyrene standards: 996-188,000 Da).

3.2.3 Ramé-Hart Contact Angle Goniometer

Hydrophilicity of the obtained products determined by air-water contact angle measurement of the polymer films were measured on a Ramé-Hart Contact Angle Goniometer (USA), model 100-00 equipped with Gilmont syringe and 24-gauge flat-tipped needle.

3.2.4 Differential Scanning Calorimeter

Differential scanning calorimetry (DSC) were carried out on DSC 204F1, NETZSCH (Germany). DSC curves were obtained by heating samples from room temperature to 240 °C at a heating rate of 10 °C/min under a nitrogen atmosphere.

3.2.5 Simultaneous Thermal Analyzer

Thermogravimetric (TG) analysis was performed on STA 449F3 Jupiter, NETZSCH (Germany). Each sample was run from room temperature to 500 °C at a scanning rate of 10 °C/min under a nitrogen atmosphere.

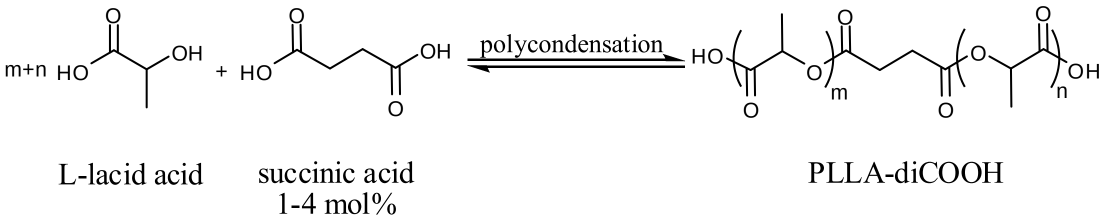
3.3 Methods

3.3.1 Synthesis of telechelic PLLAs

PLLA with dicarboxyl terminated chain ends (PLLA-diCOOH) and dihydroxyl terminated chain ends (PLLA-diOH) were polymerized via condensation reaction by using different catalysts; titanium (IV) n-butoxide (TNBT) and a binary catalyst system, $SnCl_2$•$2H_2O$ co-catalyzed by para-toluene sulfonic acid (PTSA), in order to determine an applicable catalyst for the polymerization of L-lactic acid. The former procedure using TNBT as a catalyst was carried out according to the research of Chen *et al*. 2006 [19] and Zeng *et al*. 2009 [22] while the latter using binary catalysts followed the research of Song and Wu. [20]

3.3.1.1 Synthesis of PLLA-diCOOH

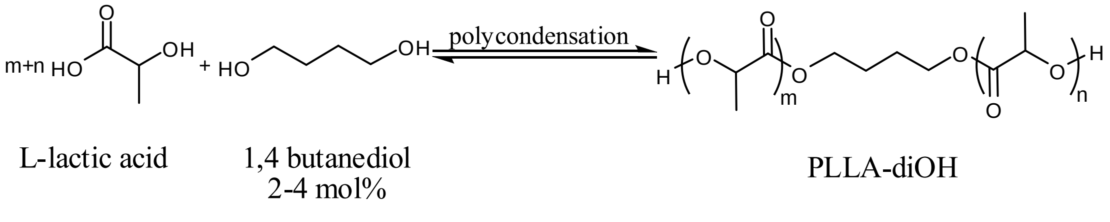
An aqueous L-lactic acid and a small amount of succinic acid (2 mol% of L-lactic acid) were added into a 50 ml three-neck round-bottom flask equipped with a condenser and a magnetic stirrer. The mixture was firstly heated at 80 °C for 2 h with slightly reduced pressure to remove free water. Next, the reaction was conducted at 160 °C for 2-5 h with further reduced pressure. After that, TNBT (0.1 mol% of L-lactic acid) was added under nitrogen atmosphere. This was followed by gradually reducing pressure until reaching high vacuum and the temperature of 180 °C with varied polymerization time of 5-9 h (Scheme 3.1). The crude product was dissolved in chloroform and precipitated in methanol. It was repeatedly precipitated 2 more times before filtration and subsequent dried under vacuum. Ultimately, white powder was obtained. For the binary catalyst system, PTSA (PTSA/Sn 1/1 mol/mol) was equally divided into 2 portions. The first portion of PTSA was added into the mixture of an aqueous L-lactic acid and various amounts of succinic acid (1-4 mol% based on number of mol of L-lactic acid). To dehydrate, the mixture was first heated at 110 °C for 1 h, subsequently heated up to 140 °C and then kept at this temperature for 1 h under slightly reduced pressure. Next, the solution was heated up to 160 °C and kept at this temperature for 2 h. $SnCl_2{\bullet}2H_2O$ (0.4 wt % of L-lactic acid) and the second portion of PTSA were subsequently added into the reaction flask under normal atmosphere. After adding the catalysts, the pressure was reduced stepwise until reaching high vacuum. The reaction was continued at the temperature of 180 °C for 4-6 h. The viscous liquid was dissolved in chloroform and precipitated in cold methanol or ethanol. After purification and dry under vacuum, white powder was collected.



Scheme 3.1 Synthesis of PLLA-diCOOH via polycondensaton.

3.3.1.2 Synthesis of PLLA-diOH

PLLA-diOH was synthesized by the same methods and conditions as the synthesis of PLLA-diCOOH, while varied amounts of 1,4-butanediol (2, 3, and 4 mol% based on mol of L-lactic acid) were added instead of succinic acid (Scheme 3.2) to produce PLLA having dihydroxyl group on both chain ends of the polymer.



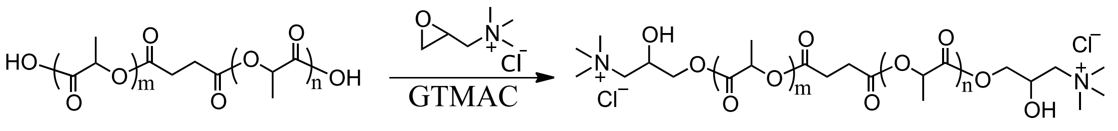
Scheme 3.2 Synthesis of PLLA-diOH via polycondensation.

3.3.2 Synthesis of positively charged PLLA

After PLLA-diCOOH and PLLA-diOH were obtained by polycondensation, positively charged PLLAs were then synthesized via nucleophilic substitution reaction between PLLA-diCOOH or PLLA-diOH and GTMAC which contains a positively charged quaternary ammonium producing PLLA-diCOOH+ and PLLA-OH+, respectively.

3.3.2.1 Synthesis of PLLA-diCOOH+

PLLA-diCOOH and an excess amount of GTMAC (2, 3, or 4 equiv. compared to the number of chain ends of the polymer reactant) were dissolved in different solvents (DMF or DMSO) (Scheme 3.3). The substitution reaction was carried out in 25 ml round-bottom flask equipped with a magnetic stirrer at different temperatures (60, 70, or 80 °C) for 24 h. The crude product was precipitated in 2 M NaCl aqueous solution and was then collected after centrifugation at 4,000 rpm. The polymer was rinsed with de-ionized water three times and was collected after centrifugation at 4,000 rpm, subsequently dried by freeze-drying. Consequently, white powder was obtained. Additionally, to increase the degree of substitution reaction, triethylamine was added to be a catalyst.

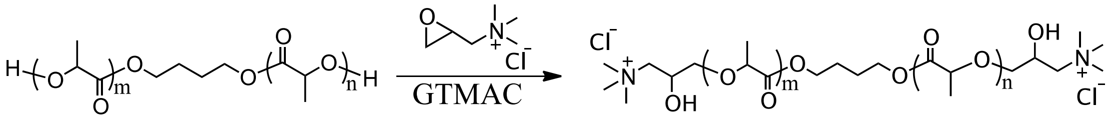


PLLA-diCOOH p-PLLA-diCOOH

Scheme 3.3 Synthesis of PLLA-diCOOH+.

3.3.2.2 Synthesis of PLLA-diOH+

PLLA-diOH+ was synthesized by the same procedure as the synthesis of PLLA-diCOOH+. Briefly, the mixture of PLLA-diOH and GTMAC in DMSO were stirred at desired temperature for 24 h (Scheme 3.4). After purification and freeze-drying, white powder of polymer was obtained.



PLLA-diOH p-PLLA-diOH

Scheme 3.4 Synthesis of PLLA-diOH+.

3.3.3 Film preparation

For contact angle measurement, blending films were prepared. The neat films of the synthesized polymers in this work couldnot be prepared because their Mw was too low. Therefore, the positively charged PLLA were blended with commercial PLLA

($\overline{M}_w$ = 100,000 Da from Nature Works) as 20, 30, and 40% of the modified polymers in total. The mixtures were dissolved in chloroform and then left at room temperature for 3 days on glass slides, the polymer films were obtained.

3.3.4 Hydrolytic degradation test

Hydrolytic degradation of the synthesized polymers were investigated by immersion of the polymer powder into pH 7.4 phosphate buffer solution according to the research of Liu *et al*. 2007 [11]. Approximately 30 mg of powdered polymers were put into glass bottles with screw caps. After adding 5 mL of pH 7.4 phosphate buffer solution, the bottles were sealed and placed in an oven at 37 °C. At desired times, the bottles were removed from the oven. Next, the mixtures were centrifuged, and the collected polymer powder was rinsed repeatedly with de-ionized water 3 times. The powder samples were freeze-dried. Finally, changes in molecular weight of the polymers were analyzed by GPC.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter was divided into three parts. The first part is the synthesis of telechelic PLLAs, PLLA-diCOOH and PLLA-diOH, which were synthesized through condensation polymerization. Secondly, the chain ends of the polymers were altered via substitution reactions between their chain ends, -COOH or -OH and a reactive epoxide of GTMAC. GTMAC consists of a positively charged quaternary ammonium; therefore, positively charged PLLAs were obtained. In the last part, hydrophilicity, hydrolytic degradation, and thermal properties of the obtained polymers were determined.

4.1 Synthesis and Characterization of telechelic PLLAs

PLLA-diCOOH and PLLA-diOH were synthesized via polycondensation by using each of two catalysts, TNBT or $SnCl_2$/PTSA. The polymers were characterized by ^1H NMR and GPC. Consequently, efficiency of both polymerization catalysts were compared.

4.1.1 Synthesis and Characterization of PLLA-diCOOH

As shown in Scheme 3.1 (Chapter III), a small amount of succinic acid (1-4 mol%), a dicarboxylic acid, was used to be an initiating point of the esterification of lactic acid in order to synthesize PLLA with two carboxylic end groups (PLLA-diCOOH). After polymerization, viscous liquid was purified by dissolving the crude product in chloroform. PLLA-diCOOH was precipitated in cold methanol or ethanol before dried under high vacuum. Percentage yield of the white powder polymer was calculated based on weight ratio of dried sample to theoretical weight as shown in Eq. 1.

$$\%yield = \frac{weight\ of\ dried\ polymer\ (g)}{(mol\ of\ lactic\ acid\ x\ 72)+(mol\ of\ succinic\ acid\ x\ 84)}\ \text{x}\ 100 \qquad \text{(Eq. 1)}$$

According to ^1H NMR spectra (Figure 4.1), the proton signals at 1.58 and 5.18 ppm were assigned to methyl (b) and methine (a) protons of the lactyl unit, respectively. The methylene proton signals (c) of succinic acid in PLLA-diCOOH chain appeared at 2.75 ppm. The number average molecular weight ($\overline{M_n}$) of the polymer could be determined from the relative intensities of proton signals in NMR spectra (Eq. 2).

$$\overline{M_n}\, of\ PLLA - diCOOH = (72\, x \frac{^aH}{^cH/4}) + 178 + 84 \qquad \text{(Eq. 2)}$$

^aH, ^cH = Intensity of methine protons of the lactyl unit, methylene proton of succinic acid, respectively

However, undesired PLLA which has one hydroxyl and one carboxyl group on its chain ends was also observed by the presence of low intensity signal at 4.36 ppm, which belonged to the methine protons in the hydroxyl end unit (Figure 4.2) in the product obtained after low (1 mol%) content of the succinic acid was used in the polymerization (Figure 4.2). This suggests that low amount of succinic acid provides less opportunity of succinic acid to condense with lactic acid. To suppress this undesired product, catalyst type and polymerization time were adjusted.

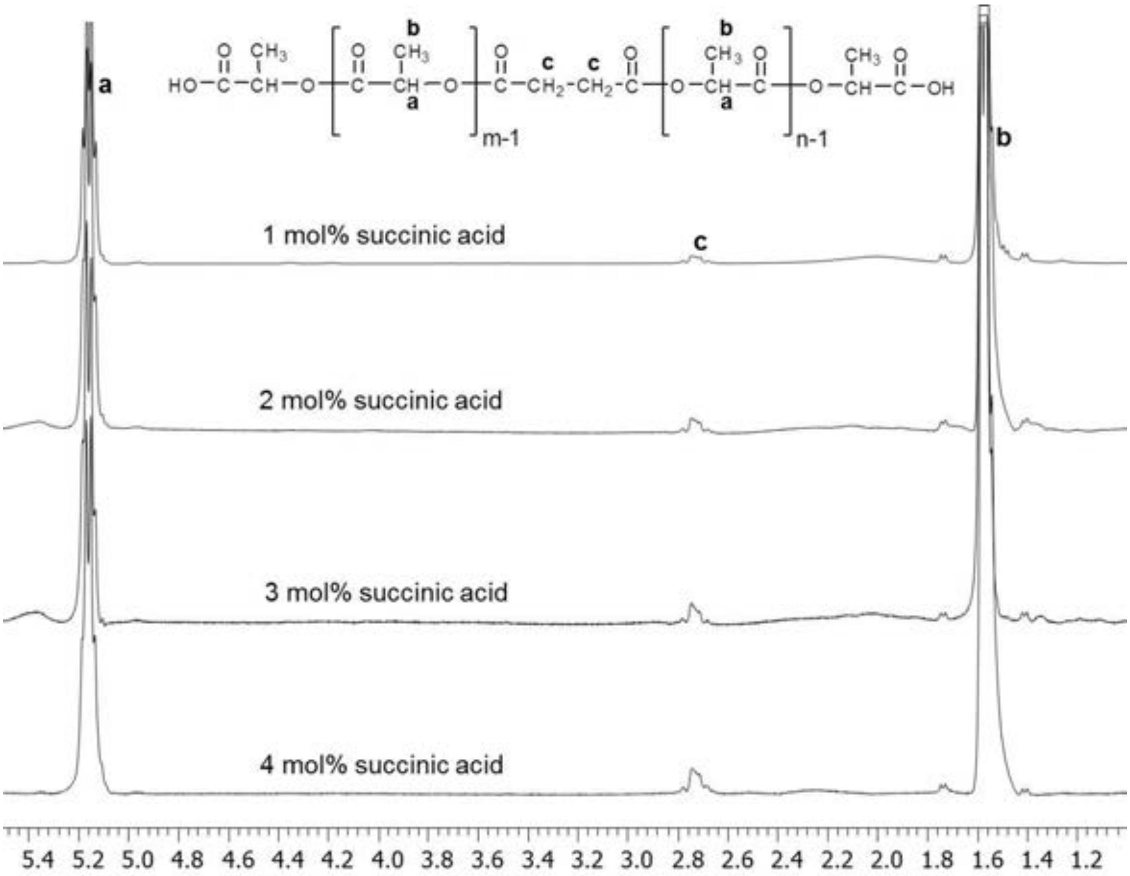


Figure 4.1 ^{1}H NMR spectrum (in $CDCl_3$) of PLLA-diCOOH with different amounts of succinic acid.

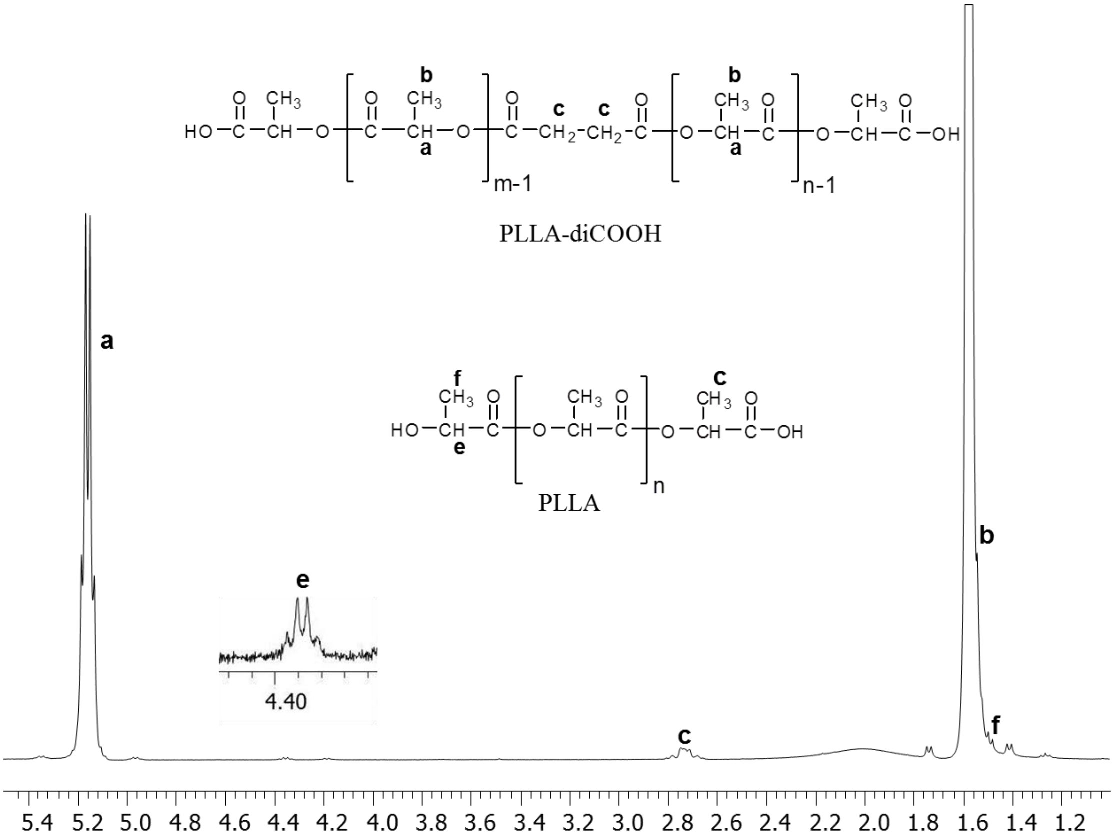


Figure 4.2 PLLA appeared in ^{1}H NMR spectrum of PLLA-diCOOH containing 1 mol% of succinic acid in $CDCl_3$.

When TNBT was used as a catalyst and 2 mol% of succinic acid was added to synthesize the dicarboxyl polymer (Table 4.1), the yield was quite low. After increasing the oligomerization time (at 160 °C) from 2 to 5 h, the yield increased. This indicates the importance of oligomerization stage to polycondensation because the carboxyl end groups at high concentration reportedly was able to reduce the catalyst efficiency for activating reaction sites [1]. Although both oligomerization and polymerization time were increased, normal PLLA chain still remained (revealed by the proton signals at δ4.36 and δ1.48 that were methine and methyl proton of the terminal lactyl unit on the hydroxyl end side). The relative amount of undesired PLLA was determined based on the ratio of number of PLLA chains to number of all polymer chains as shown in Eq. 3.

$$\%PLLA = \frac{e_H}{e_{H} + c_{H/4}} \text{x } 100 \qquad \text{(Eq. 3)}$$

Table 4.1 Effect of oligomerization and polymerization time on percentage yield and normal PLLA in the synthesis of PLLA-diCOOH using TNBT catalyst and 2 mol% of succinic acid

Entry	Time (h)		%Yield	^{1}H NMR		GPC	
	Oligomerization (160 ºC)	Polymerization (180 ºC)		%PLLA	$\overline{M}_n$	$\overline{M}_n$	PDI
1	2	5	25	23	3,845	4,668	1.18
2	2	7	38	29	3,785	5,714	1.18
3	2	9	25	29	5,360	6,818	1.19
4	5	9	52±4	15±2	4,874	5,714	1.18

After replacing TNBT by $SnCl_2$ and PTSA as a binary catalyst system (Table 4.2), the PLLA-diCOOH yield increased. This means more efficient condensation reaction facilitated by the binary catalyst due to addition of a strong protonic acid like PTSA as co-catalyst which can stimulate the dehydration as explained in Chapter 2. The molecular weight of PLLA-diCOOH determined by ^{1}H NMR and GPC analysis were in the range of 2,700-8,800 Da depending on the added amount of succinic acid. Increasing in mol% of succinic acid lowered $\overline{M}_n$ of the polymers because more number of the polymer chains was obtained, therefore, the number of repeating units per a polymer chain decreased. When 3 and 4 mol% of succinic acid were added, normal PLLA couldn't be detected by ^{1}H NMR (no proton signal at at $\delta 4.36$ and $\delta 1.48$ ppm) while adding 1 and 2 mol% of succinic acid those proton signals were present. This can be explained the fact that increasing amount of succinic acid provides more chance of succinic acid molecules to combine together with the lactic acid monomer and/or the PLLA chains, so the formation of PLLA chain is suppressed. Moreover, polymerization time seems to affect the presence of normal PLLA. There was no proton signals detected for sample 2b which was polymerized for 6 h but for sample 2a (polymerized for 4 h), those signals still appeared. Increase in polymerization time using $SnCl_2$/PTSA can eliminate the unwanted PLLA. Using cold EtOH to precipitate the product (sample 4b) gave higher recovery amount than using cold MeOH (sample 4a) (Table 4.3). It is because EtOH is slightly less polar than MeOH, thereby EtOH dissolve less amount of low molecular weight polymer. PLLA-diCOOH synthesized by using 2 mol% of succinic acid and 6 h for polymerization time was chosen for the next reaction step (with GTMAC) since this reaction condition gave acceptable molecular weight and no PLLA.

The reason why $\overline{M}_n$ of the polymers obtained from both ^{1}H NMR and GPC analysis were larger than that from theoretical calculation may be because not all the diacids or diols added into the reaction (in feed) could combine with the polymer chains. Only some of them appeared in the chains, less number of PLLA-diCOOH or PLLA-diOH chains were formed. This led to PLLA chains with higher number of lactyl units than expected.

Table 4.2 Effect of amount of succinic acid and polymerization time on percentage yield and normal PLLA in the the synthesis of PLLA-diCOOH using $SnCl_2$/PTSA catalyst

Entry	Mol% of succinic acid	%Yield	Theoretical	^{1}H NMR		GPC	
			$\overline{M}_n$ (Da)	%PLLA	$\overline{M}_n$ (Da)	$\overline{M}_n$ (Da)	PDI
1	1	77±3	7,462	24±4	7,957±368	8,809±973	1.42±0.02
2a	2	68±4	3,862	16±1	4,735±387	6,153±621	1.29±0.02
2b	2	66±5	3,862	ND[1]	5,168±410	6,566±747	1.29±0.02
3	3	62±3	2,662	ND	4,071±324	5,828±270	1.23±0.01
4a	4	49±4	2,062	ND	3,500±40	5,029±151	1.19±0.01
4b	4	59±2	2,062	ND	3,243±341	5,109±73	1.19±0.03

[1] ND means %PLLA couldn't be detected by ^{1}H NMR (no proton signal at δ4.36).

- Sample 1, 2a, 3, 4a, and 4b were polymerized for 4 h while 2b was polymerized for 6 h.
- Sample 4a and 4b were precipitated in cold MeOH and EtOH, respectively.

To prove that some polymers can dissolve in the alcohol used as precipitating solvent, the filtrate of each samples after precipitation was collected, left the solvent to evaporate at room temperature first, and subsequently dried under high vacuum. The residue was weighed and analyzed by GPC (Table 4.3). Polymers with low $\overline{M_n}$ in the range of 2,400-3,000 Da were found soluble in the precipitating solvent. This therefore resulted in low recovery of the products. Nevertheless EtOH should be more appropriate for polymer purification than MeOH. It should be noted here that the percentage recovery value were less than 100, this may be caused by the sublimation of lactic acid out of the reaction flask during the reaction at high temperature.

Table 4.3 Effect of precipitating solvent on percent yield of PLLA-diCOOH

Entry	Mol% of succinic acid	$\overline{M_n}^{\text{GPC}}$ of product	%Yield	%Weight of residue	%Recovery	$\overline{M_n}^{\text{GPC}}$ of residue
1	1	8,730	75	13	88	1,735
2	2	6,738	72	19	91	2,427
3	3	5,934	62	28	90	3,039
4a	4	4,872	49	42	91	2,414
4b	4	5,521	61	34	95	2,487

4.1.2 Synthesis and Characterization of PLLA-diOH

A small amount of 1,4-butanediol (2-4 mol%) was used to be a starting material to react with lactic acid in order to synthesize PLLA-diOH as shown in scheme 3.2. White powder polymer was obtained, with the yield calculated as shown in Eq. 4:

$$\%yield = \frac{weight\ of\ dried\ polymer\ (g)}{(mol\ of\ lactic\ acid\ x\ 72)+(mol\ of\ butanediol\ x\ 88)}\ \text{x}\ 100 \qquad \text{(Eq. 4)}$$

The chemical structure of dihydroxyl terminated PLLA (PLLA-OH) was characterized by ^{1}H NMR technique (Figure 4.3). The signals of protons belonging to the inner and outer methylene groups of butanediol appeared at δ1.69 (d) and δ4.15

(c) ppm, respectively. The peaks at $\delta5.18$ (a) and $\delta1.58$ (b) ppm were assigned to methine and methyl proton in the repeating unit, respectively. The peaks observed $\delta1.48$ (b') and

δ 4.36 (a') belonged to the corresponding protons at the terminal lactyl unit on the hydroxyl chain end. The number average molecular weight ($\overline{M}_n$) were calculated from the NMR spectrum as shown in Eq. 5:

$$\overline{M}_n\, of\ PLLA - diOH = (72\, x \frac{a_H}{c_{H/4}}) + 146 + 88 \qquad \text{(Eq. 5)}$$

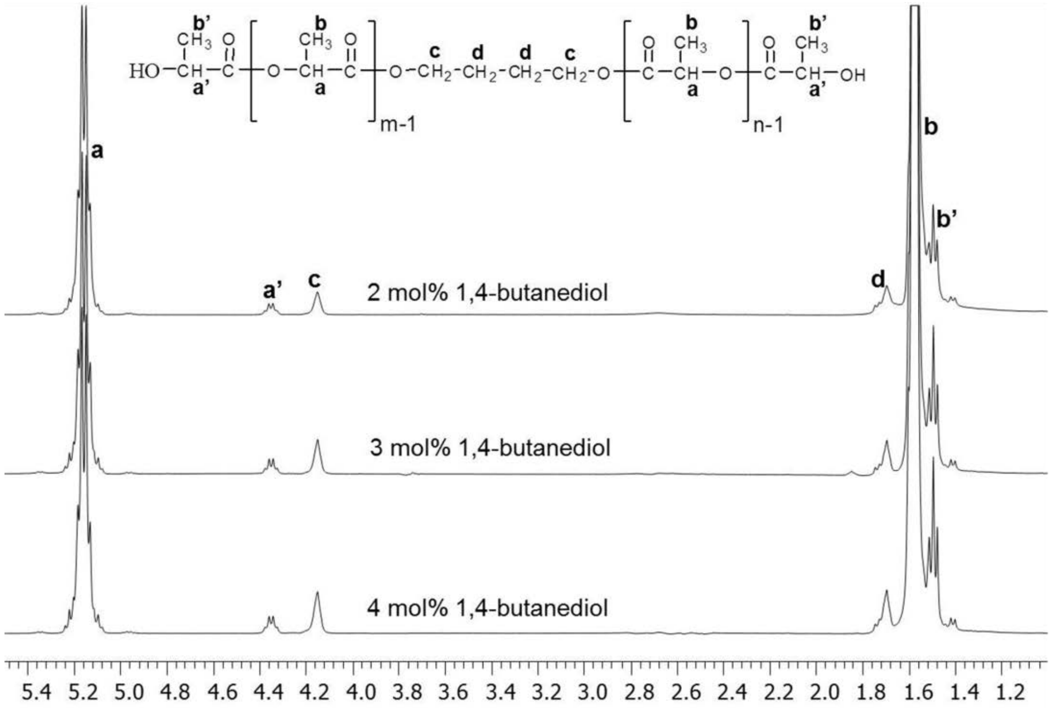


Figure 4.3 ^{1}H NMR spectrum of PLLA-diOH in with different amounts of 1,4-butanediol in $CDCl_3$.

The percentage yield of the polymer was quite low when using TNBT as a catalyst (Table 4.4) but it became higher with increasing oligomerization time as also observed for PLLA-diCOOH. Oligomerization period of 5 h and polymerization period of 6 h was able to suppress the unwanted PLLA (performed by integral ratio of proton signal at $\delta4.15$ (c) to $\delta4.36$ (a') must be 2). The % remaining PLLA for sample 1 and 2 (Table 4.4) were determined by Eq. 6.

$$PLLA = \frac{{}^{a'}H-({}^{c}H/2)}{({}^{a'}H-({}^{c}H/2))+{}^{c}H/4} \text{x } 100 \qquad \text{(Eq. 6)}$$

Table 4.4 Effect of oligomerization and polymerization time on percentage yield and normal PLLA in the the synthesis of PLLA-diOH using TNBT catalyst and 2 mol% of 1,4-butanediol

Entry	Time (h)		%Yield	^{1}H NMR		GPC	
	Oligomerization (160 ºC)	Polymerization (180 ºC)		%PLLA	$\overline{M_n}$	$\overline{M_n}$	PDI
1	-	5	22	33	3,695	5,119	1.18
2	2	9	32	44	4,806	3,269	1.14
3	5	9	54	ND[1]	4,849	6,429	1.18
4	5	9	53	ND	4,986	7,592	1.28

[1] ND, not detected means %PLLA couldn't be detected by ^{1}H NMR analysis.

As illustrated in Table 4.5, using $SnCl_2$/PTSA as catalysts, the yield was higher than using TNBT. Thus the binary catalysts was more efficient in the condensation polymerization of both PLLA-diOH and PLLA-diCOOH. The molecular weight of PLLA-diOH determined by ^{1}H NMR and GPC were in the range of 3,000-7,600 Da depending on the added amount of 1,4-butanediol. Increasing in mol% of 1,4-butanediol lowered $\overline{M_n}$ of the polymers as earlier explained. The condition of 2 mol% of 1,4-butanediol and 6 h for polymerization time was applicable to synthesize PLLA-diOH and the polymer was then utilized to react with GTMAC in order to produce positively charged PLLA (PLLA-diOH+).

Table 4.5 Effect of amount of succinic acid and polymerization time on percentage yield and normal PLLA in the the synthesis of PLLA-diOH using $SnCl_2$/PTSA catalyst

Entry	mol% of 1,4-butanediol	%yield	^{1}H NMR	GPC	
			$\overline{M}_n$	$\overline{M}_n$	PDI
1	2	71±3	5,065±108	6,571±1,147	1.30±0.02
2	3	55±9	3,757±169	5,698±448	1.22±0.01
3	4	50±3	3,097±102	5,198±406	1.18±0.02

Sample 1 was polymerized for 6 h, while 2 and 3 were polymerized for 4 h.

4.2 Synthesis and Characterization of positively charged PLLA

The chain ends of PLLA-diCOOH and PLLA-diOH were altered from –COOH or -OH to positively charged quaternary ammonium by reacting with GTMAC as shown in Scheme 3.3 and 3.4 (Chapter III). When the obtained polymers were purified and dried by freeze dryer, white powder of each sample was collected to determine percentage yield as shown below:

$$\%yield = \frac{weight\ of\ dried\ polymer\ (g)}{(mol\ of\ lactic\ acid\ x\ 72)+(mol\ of\ succinic\ acid\ x\ 84)}\ \text{x}\ 100 \qquad \text{(Eq. 7)}$$

After substitution of GTMAC at the polymer chain ends, the methyl proton signal of GTMAC appeared at δ3.38 ppm. The relative intensity of this proton signal of PLLA-COOH+ (Figure 4.4) was higher than that of PLLA-OH+ (Figure 4.5), indicating higher degree of substitution in PLLA-COOH+. In addition, a low-intensity proton signal at δ4.36 ppm (d, Figure 4.4) which was the methine proton at the hydroxyl chain end in PLLA surprisingly appeared after the substitution reaction. This unexpected finding suggested that a small number of ester bonds in the polymer chains were hydrolyzed. It was possible that traces of water remained in the GTMAC (Figure A.9) even after attempts to dehydrate the reagent under high vacuum. Therefore, the polymer chain was attacked by water molecule causing chain cleavage.

Degree of substitution reaction (%DS) of PLLA-COOH+ (Eq. 8) and PLLA-OH+ (Eq. 9) were calculated based on the ratio of number of GTMAC appearing in polymer chain to total number of chain ends.

$$\%DS\ of\ PLLA - diCOOH+ = \frac{{}^{h}H/9}{2({}^{d}H)+({}^{c}H/2)} \text{x } 100 \qquad \text{(Eq. 8)}$$

$$\%DS\ of\ PLLA - diOH+ = \frac{{}^{h}H/9}{2({}^{a'}H - {}^{c}H/2)+({}^{c}H/2)} \text{x } 100 \qquad \text{(Eq. 9)}$$

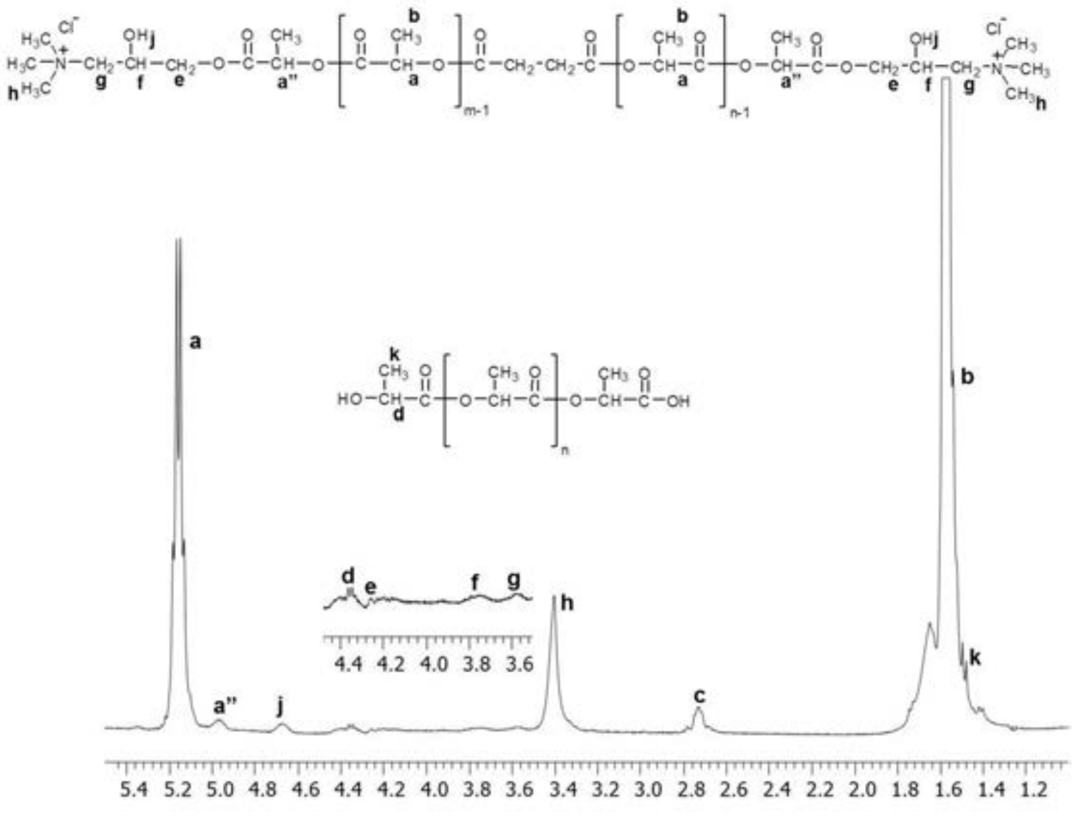


Figure 4.4 ^{1}H NMR spectrum of PLLA-diCOOH+ in CDCl$_3$.

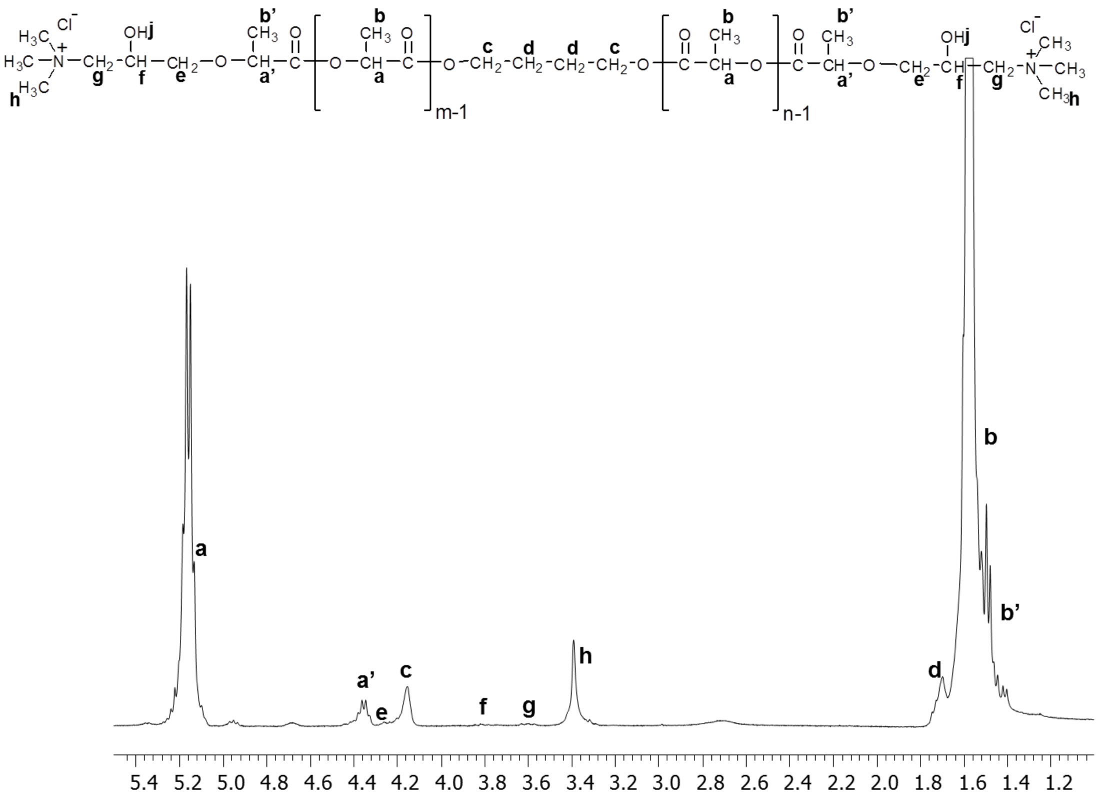


Figure 4.5 ^{1}H NMR spectrum of PLLA-diOH+ in $CDCl_3$.

4.2.1 Effect of amount of GTMAC on degree of substitution

The reaction was carried out by stirring the mixture of polymer and GTMAC, in DMSO at 80 ºC for 24 h. When using 3 equiv. GTMAC, %DS was the highest. Therefore, 3 equiv. GTMAC should be an optimal amount to synthesize the positively charged PLLA. From Table 4.6, it can be seen that increasing the amount of GTMAC in the reaction caused the yield and $\overline{M_n}$ to decrease. This can be explained by free water in GTMAC couldn't be completely removed by high vacuum; thereby, adding more amount of GTMAC more water molecules contaminated into the reaction causing more ester bond hydrolysis.

Table 4.6 Characteristic of PLLA-diCOOH+[a] synthesized with various amount of GTMAC

entry	[b]GTMAC (equiv.)	%yield	%DS	^{1}H NMR	
				$\overline{M}_n$	[c]% $\Delta\overline{M}_n$
1	2	91±5	72 ± 3.3	4,812±156	-1
2	3	82±1	84 ± 2.8	4,121±128	-15
3	4	51±9	83± 3.1	3,293±313	-35

[a]Synthesized from PLLA-COOH with $\overline{M}_n$ = 4,841 Da and carried out in DMSO at 80 ºC for 24 h

[b]Amount of GTMAC (equiv.) was compared with number of mol of polymer chain ends.

[c]Percentage difference between $\overline{M}_n$ of the starting PLLA-diCOOH and the resulting PLLA-diCOOH+.

4.2.2 Effect of reaction temperature on degree of substitution

The mixture of polymer and 4 equiv. GTMAC were stirred in DMSO at 60, 70, and 80 ºC for 24 h. The results in Table 4.7 showed that the yield and $\overline{M}_n$ decreased with increasing reaction temperature. The degree of substitution was insignificantly different within the temperature range from 60-80 ºC probably because an excess amount of GTMAC was used (4 equiv.). However, degree of substitution at 70 ºC should be optimum due to providing the highest %DS.

Table 4.7 Characteristic of PLLA-diCOOH+[a] synthesized at different temperatures

entry	Temperature (°C)	%yield	%DS	^{1}H NMR	
				$\overline{M}_n$	[b]% Δ $\overline{M}_n$
1	60	65±8	81±0.7	3,813±283	-28
2	70	60±9	84±1.1	3,561±114	-24
3	80	51±9	83±3.1	3,293±313	-35

Stirred in DMSO, 4 eq. GTMAC, 24 h

[a] Synthesized from PLLA-COOH with M_n = 4,841 Da.

[b] % difference between $\overline{M}_n$ of the starting PLLA-diCOOH and the resulting PLLA-diCOOH+.

4.2.3 Effect of catalyst and polarity of solvent on degree of substitution

In this section, the reactivities of carboxyl and hydroxyl end groups in PLLA-diCOOH and PLLA-diOH, respectively, were compared. The reactions were carried out at 70 °C in DMSO or DMF.

The degree of substitution in PLLA-COOH+, synthesized from PLLA-COOH was considerably higher than that obtained from PLLA-OH (Table 4.6 and 4.7). This is explained by the fact that carboxylate form of PLLA-COOH has more reactivity than the hydroxyl group of PLLA-OH. In another test, when DMSO was used as a solvent, it showed a slight increase in the degree of substitution because of higher polarity of DMSO compared to DMF, appropriate for nucleophilic substitution reaction. Moreover adding triethylamine as a catalyst helped increase the degree of substitution from 80 to 90% (in DMSO). This was because triethylamine acted as a base to abstract acidic proton of the carboxyl group in PLLA-COOH to become a more active carboxylate ion. The reaction of PLLA-OH and GTMAC in the presence of the catalyst showed the similar results of %DS because NEt_3 was not able to increase the reactivity of its hydroxyl end group.

Table 4.8 Effect of NEt_3 and solvent on degree of substitution of PLLA-diCOOH+[a]

entry	solvent	NEt_3	%yield	%DS	^1H NMR		GPC	
					$\overline{M}_n$	$^b\% \Delta \overline{M}_n$	$\overline{M}_n$	$^b\% \Delta \overline{M}_n$
1	DMF	-	70±6	68±0.7	4,561	-7	1,016, 5,320	-85, -20
2	DMF	1 eq.[c]	65±2	73±2.0	4,578	-7	720, 4,510	-89, -32
3	DMSO	-	73±4	80±1.5	4,403	-10	3,906±360	-41
4	DMSO	1 eq.	74±3	90±0.8	4,602	-6	3,906±6	-41

3 eq. GTMAC, 70 ºC, 24 h, repeated 3 times

[a] Synthesized from PLLA-diCOOH with $\overline{M}_n^{NMR}$ = 4,899 Da, $\overline{M}_n^{GPC}$ = 6,642 Da

[b] %difference between $\overline{M}_n$ of the starting PLLA-diCOOH and the resulting PLLA-diCOOH+.

[c] compared with mol of polymer end groups.

Table 4.9 Effect of NEt_3 on degree of substitution of PLLA-diOH+[a]

entry	solvent	NEt_3	%yield	%DS	^1H NMR		GPC	
					M_n	$^b\% \Delta M_n$	M_n	$^b\% \Delta M_n$
1	DMSO	-	59±2	19±0.4	4,132	-18	3,575±40	-43
2	DMSO	1 eq.	58±1	18±1.5	4,144	-18	3,526±64	-44

3 eq. GTMAC, 70 ºC, 24 h, repeated 3 times

[a] Synthesized from PLLA-diOH with $\overline{M}_n^{NMR}$ = 5,065 Da, $\overline{M}_n^{GPC}$ = 6,287 Da

[b] % difference between $\overline{M}_n$ of the starting PLLA-diOH and the resulting PLLA-diOH+.

In addition, when GTMAC was added into the reaction to synthesize the positively charged PLLA, the polymer molecular weight decreased due to water contamination causing hydrolysis. In a comparison study, PLLA-diCOOH and PLLA-diOH were heated without GTMAC at 70 ºC for 24 h. There was no significant change in $\overline{M}_n$ of both polymers as determined by GPC technique (Figure 4.8). And these results were also confirmed by ^{1}H NMR (see appendix, Figure A.7 and A.8).

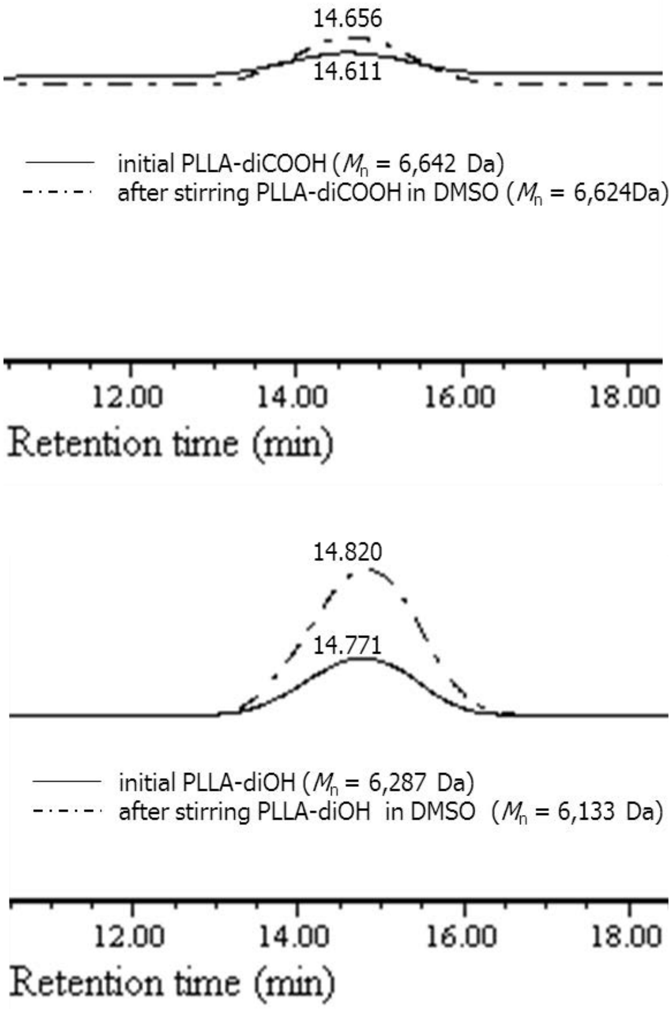


Figure 4.6 GPC trace of of PLLA-diCOOH and PLLA-diOH stirred in DMSO without GTMAC at 70 ºC for 24 h.

4.3 Polymer properties

4.3.1 Hydrophilicity test

Hydrophilicity of the modified PLLAs was determined by air-water contact angles measurement (Figure 4.8). The contact angles of the positively charged PLLAs blended with the commercial PLLA in different percentage were lower than those of the unaltered polymers, PLLA-diCOOH. Moreover, higher blending ratios have more effect on reducing the contact angle. These results support that hydrophilicity of the altered polymers are successfully enhanced.

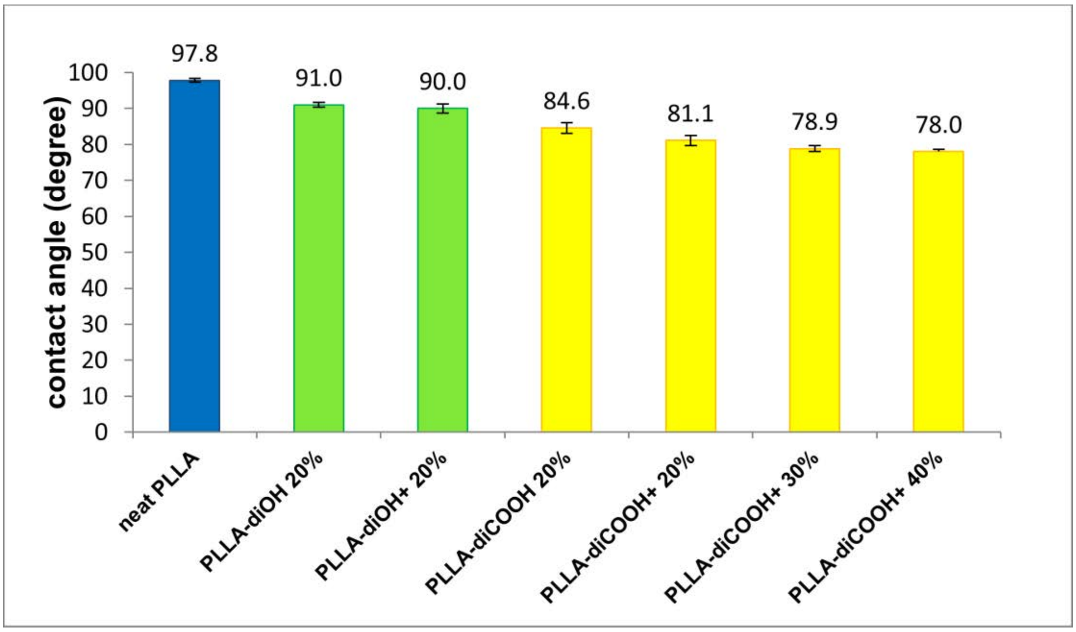


Figure 4.7 Air-water contact angle measurement of PLLA-diCOOH+ blended with commercial PLLA in different weight percentage and compared with PLLA-diCOOH (blended with the commercial PLLA 20%), and neat PLLA. PLLA-diOH+ and PLLA-diOH blending film were also determined.

4.3.2 Hydrolytic degradation

The polymer powder samples were aged in phosphate buffer without stirring. At predetermined time intervals, polymer powder of each samples were collected and dried by freeze-drying and then were analyzed by GPC. Molecular weight data measured during degradation are given in Table 4.7. GPC traces of PLLA-COOH and PLLA-COOH+ showed development of a low molecular weight shoulder after two weeks of degradation (Figure 4.9), which eventually evolved into a bimodal molecular weight distribution after the 4th week. The chromatogram of PLLA-OH and PLLA-OH+ remained as monomodal distribution. The molecular weight of most samples except PLLA-OH+ tended to decrease with increasing the aging period, especially PLLA-diCOOH which degraded with the highest rate. The highest degradation rate of PLLA-diCOOH was probably resulted from its chain ends consisting of acidic protons which could partially dissociate, causing the mixture to become more acidic. This increased in acidity could therefore induce the degradation of the polymer.

Table 4.10 Molecular weight change during hydrolytic degradation of the synthesized polymers

degradation time (week)	PLLA-diCOOH		PLLA-diOH		PLLA-diCOOH+		PLLA-diOH+	
	$\overline{M}_n$	PDI	$\overline{M}_n$	PDI	$\overline{M}_n$	PDI	$\overline{M}_n$	PDI
0	6,642	1.31	6,287	1.29	4,080	1.33	3,462	1.48
1	5,492	1.32	6,001	1.30	3,964	1.36	3,592	1.36
2	5,277	1.32	5,858	1.30	3,923	1.35	3,583	1.35
3	4,586	1.28	5,521	1.33	3,794	1.35	3,586	1.35
4	4,536	1.33	5,360	1.32	3,363	1.31	3,482	1.31

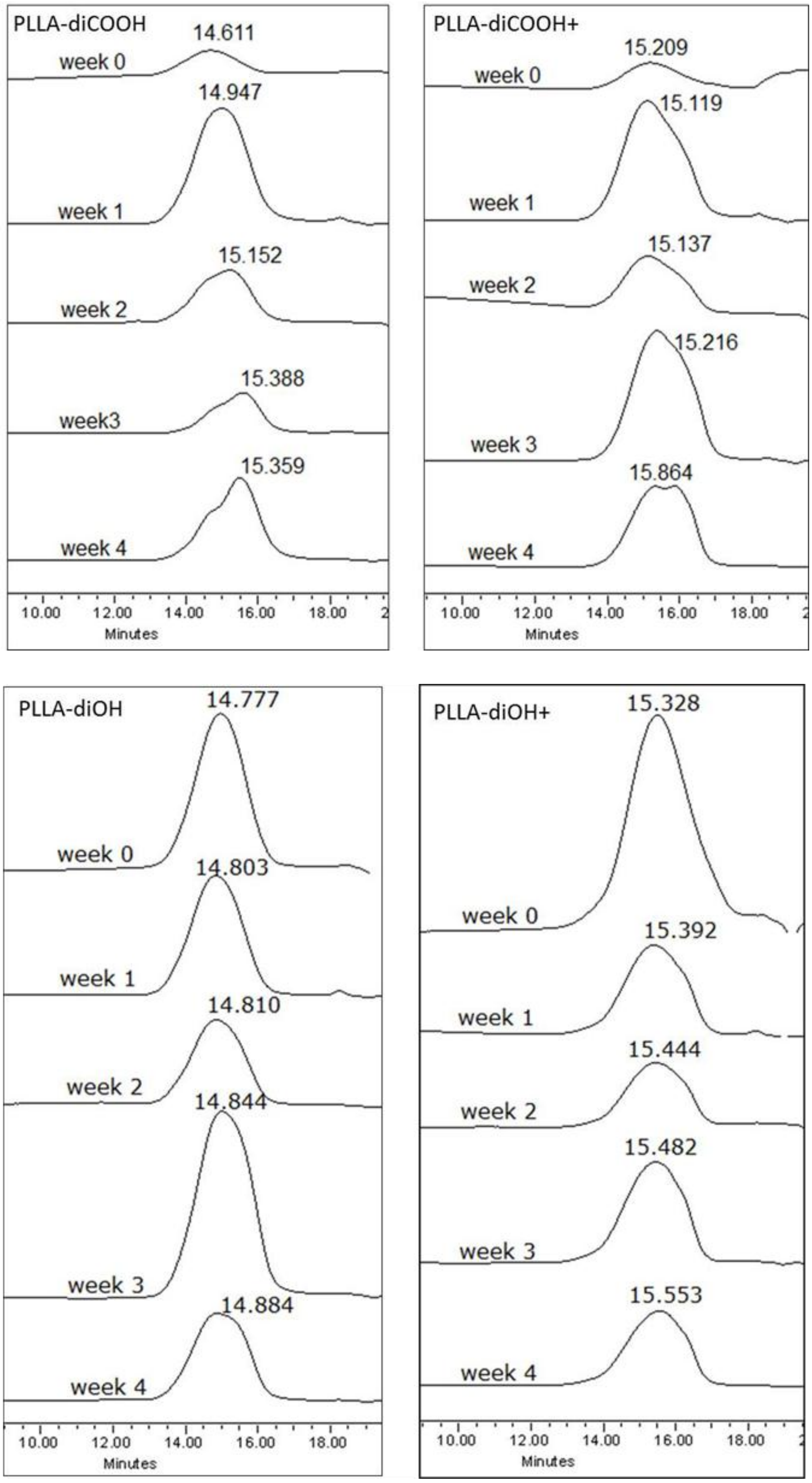


Figure 4.8 GPC traces of PLLA-diCOOH, PLLA-diCOOH+, PLLA-diOH, and PLLA-diOH+ during hydrolytic degradation at pH 7.4 and 37 °C.

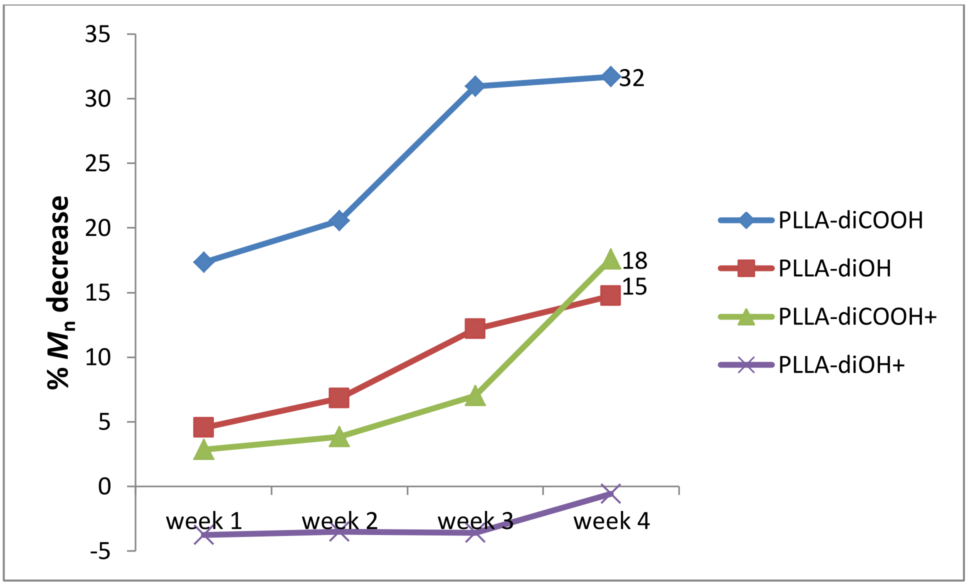


Figure 4.9 %Decreasing in $\overline{M}_n$ of PLLA-diCOOH, PLLA-diCOOH+, PLLA-diOH, and PLLA-diOH+ during hydrolytic degradation at pH 7.4 and 37 °C.

4.3.3 Thermal properties

Thermal properties of PLLA-diCOOH, PLLA-diOH, PLLA-diCOOH+, and PLLA-diOH+ with $\overline{M}_n$ (GPC) of 6,642, 6,287, 4,080 and 3,469 Da, respectively, were investigated by using DSC and TGA technique as shown in the following figures.

4.3.3.1 DSC and TGA analysis

The thermogram patterns of both positively charged PLLA are slightly different from the PLLA-diCOOH and PLLA-diOH. The small drops in Tg and Tm could be also caused by the fact that these positively charged derivatives had lower molecular weights than their parent polymers (as discussed in Section 4.2.1).

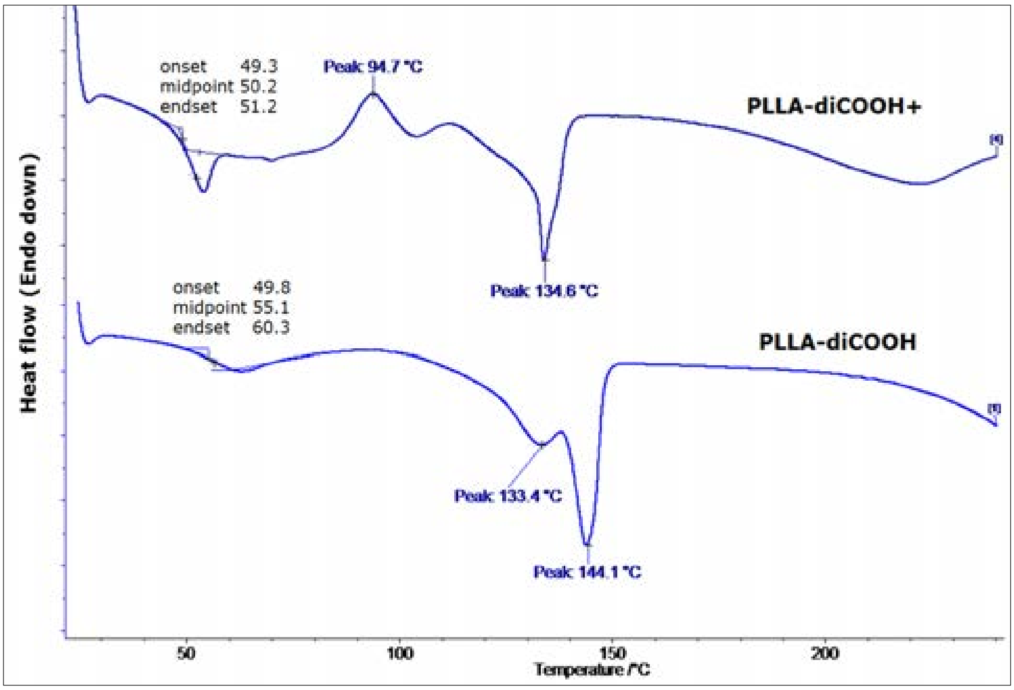


Figure 4.10 DSC trace of PLLA-diCOOH of PLLA-diCOOH+

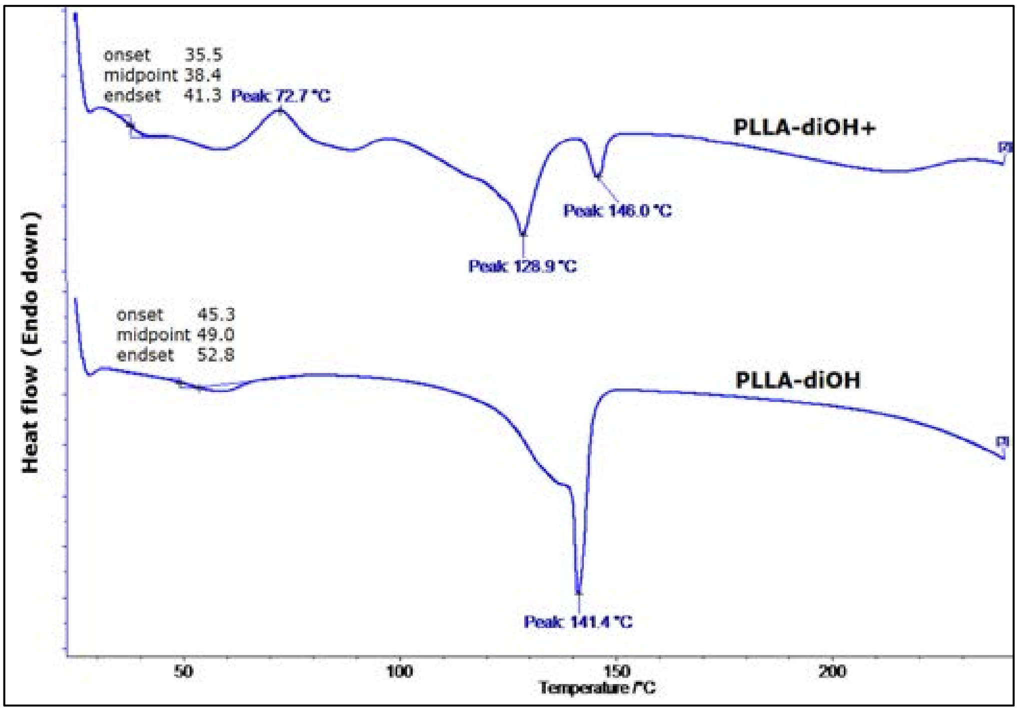


Figure 4.11 DSC thermogram of PLLA-diOH of PLLA-diOH+

As shown in TG thermogram below, the on-set decomposition temperature of positively charged PLLAs, PLLA-diCOOH+ and PLLA-diOH+ were insignificantly different from PLLA-COOH and PLLA-OH.

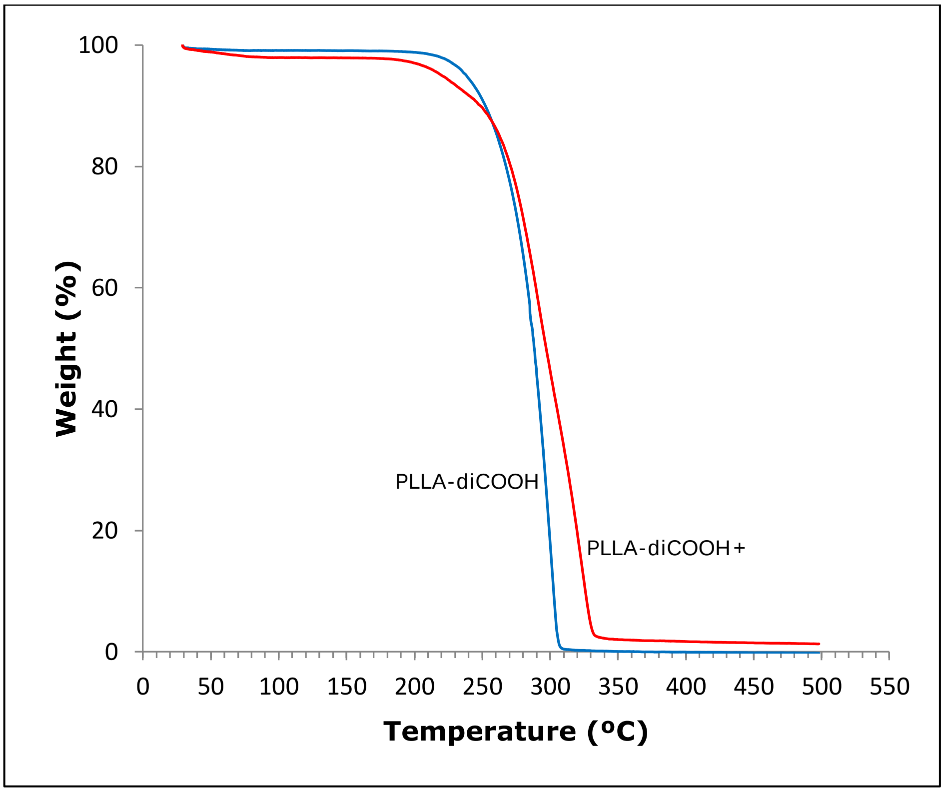


Figure 4.12 TG thermogram of PLLA-diCOOH of PLLA-diCOOH+

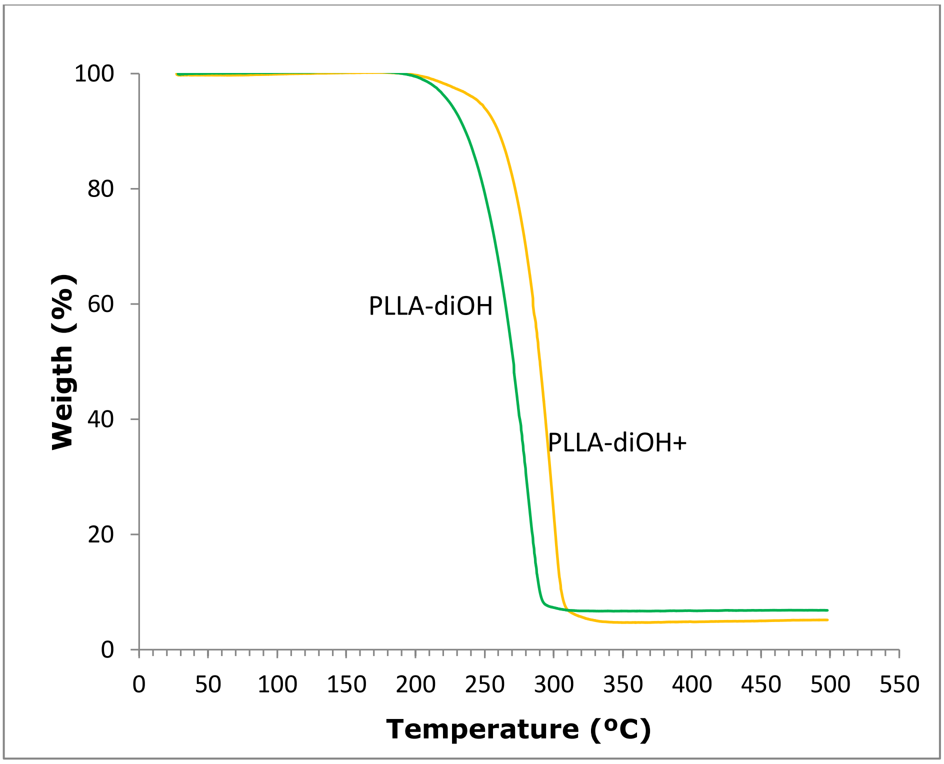


Figure 4.13 TG thermogram of PLLA-diOH of PLLA-diOH+.

Table 4.11 Data collected from TG thermogram

sample	T_{onset} (°C)	T_{max} (°C)	WL_{max} (%)
PLLA-diCOOH	267.7	304.6	99.34
PLLA-diCOOH+	260.4	328.7	96.58
PLLA-diOH	243.6	288.1	93.44
PLLA-diOH+	267.6	303.7	94.86

4.3.3.2 Thermal stability analysis

Positively charged polymer, PLLA-diCOOH+ with DS ~90% and $\overline{M}_n$ of 4,080 Da (GPC) was heated at 180 °C for 5 min ($\overline{M}_n$ = 4,024 Da) and 30 min ($\overline{M}_n$ = 3,159 Da) in order to investigate the degradation of ammonium group due to high temperature. PLA is usually molten above its T_m for melt processing (T_m ~180 °C for stereochemically pure PLA), the temperature of 180 ºC was chosen for testing the synthesized polymer, which has T_m ~135 ºC. Both ^1H NMR spectra and GPC chromatogram (Figure 4.14 and 4.15) revealed that when heating the synthesized PLA+ at 180 ºC for 5 min, the $\overline{M_n}$ remained unchanged. However after heating for 30 min at the same temperature, the ^1H NMR spectrum revealed decreasing in the intensity of methyl proton (h) of the quaternary ammonium group. This indicated the loss of ammonium group from the polymer chain due to long heating period. The GPC chromatogram also suggested an increasing amount of low molecular weight oligomers due to this long heating time.

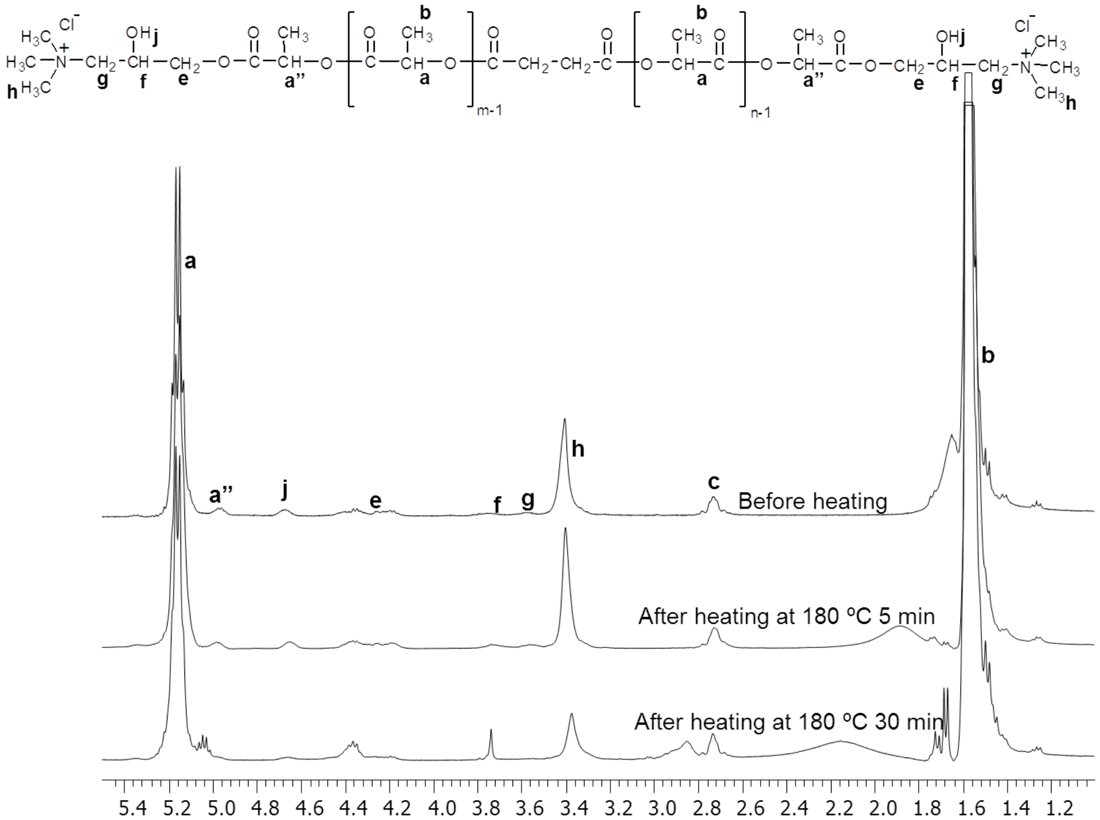


Figure 4.14 ^1H NMR spectrum of PLLA-diCOOH+ before and after heating at 180 ºC.

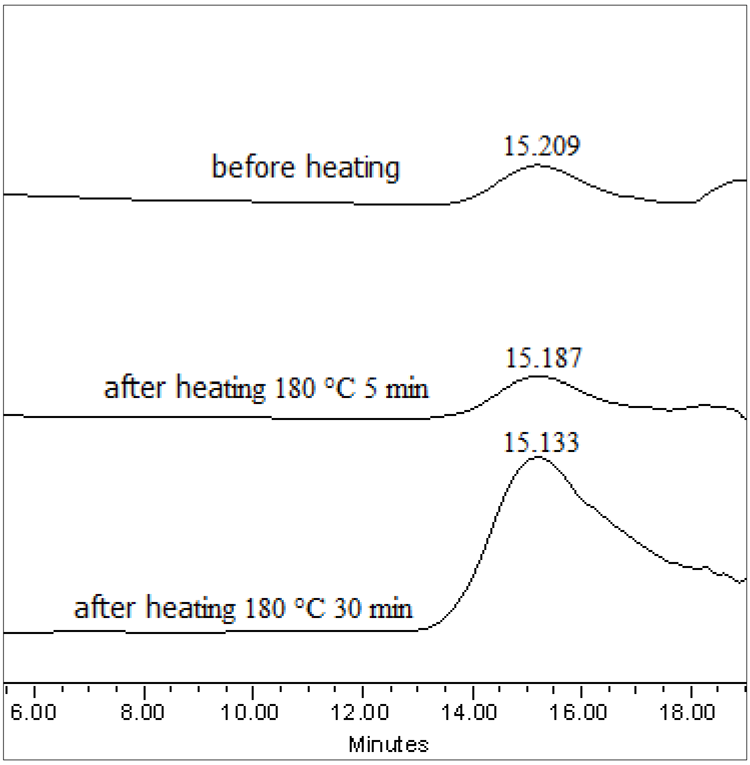


Figure 4.15 GPC trace of of PLLA-diCOOH+ before and after heating at 180 ºC.

CHAPTER V
CONCLUSION

5.1 Conclusions

Telechelic PLLAs; PLLA-diCOOH and PLLA-diOH were first synthesized via condensation polymerization. The binary catalyst system, $SnCl_2$/PTSA showed higher efficiency in increasing the product yield and better suppressing the amount of normal PLLA. When 3 and 4 mol% of succinic acid or 1,4-butanediol were used to synthesize PLLA-diCOOH and PLLA-diOH, respectively, the undesired PLLA could be suppressed while using 2 mol% of them required longer polymerization time from 4 h to 6 h to eliminate the PLLA. Nevertheless the amount of succinic acid and 1,4-butanediol of 2 mol% were chosen to prepare the telechelic polymers for synthesizing positively charged PLLA since they gave acceptable $\overline{M}_n$. The polymers obtained in the chosen condition were then utilized to react with GTMAC. Finally, the positively charged PLLAs were produced.

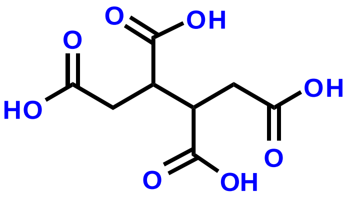
As determined by ^1H NMR, the positively charged PLLA synthesized from PLLA-diCOOH provided greater degree of substitution than that from PLLA-diOH. This is because the carboxyl (-COOH) is more reactive than hydroxyl (-OH) group in the ring-opening reaction with GTMAC due to acid catalysis ability of the -COOH group. The appropriate condition to synthesize the positively charged PLLA were stirring the mixture in DMSO at 70 ºC and using 3 equivalents of GTMAC. Additionally, using polar aprotic solvent for the nucleophilic substitution, DMSO which was more polar than DMF and adding NEt_3 as a catalyst enhanced the degree of GTMAC substitution reaction. However, the molecular weight of final positively charged PLLA slightly decreased possibly because hydrolytic degradation of polyesters took place during the reaction.

Air-water contact angle value of the positively charged PLLA blended with commercial grade PLLA in the ratio of 2:3 by weight were lower than those of PLLA-diCOOH, PLLA-diOH, and neat PLLA. Hydrolytic degradation rate of PLLA-diCOOH+ was higher than that of PLLA-diOH+ as revealed by GPC. PLLA-COOH+

eventually evolved into a bimodal molecular weight distribution after immersion the polymers in phosphate buffer for 4 weeks while PLLA-diOH+ didn't. No significant change in thermal properties after the chain ends of PLLA-diCOOH and PLLA-diOH were altered to the positively charged unit as investigated by DSC and TGA. This means bulk thermal properties of PLLA is still maintained after the chain end modification in this work.

5.2 Future Direction

In this work, only two positively charged units of quaternary ammonium group appeared on chain ends of PLLA. Therefore, in the future more carboxyl group should be available on the PLLA chain by an applicable method e.g., using a small molecule which contains several carboxyl group in a molecule such as 1,2,3,4-butanetetracarboxylic acid (1,2,3,4- BTCA) as reported in the researched of Inkinen *et al*, 2011 [23] to synthesize branched PLLA with high number of carboxyl terminated chain ends and thereby react with GTMAC to reach high number of the positively charged groups. It is believed that hydrolytic degradation rate of PLLA will dramatically increase.



1,2,3,4- BTCA

REFERENCES

[1] Maharana, T., B. Mohanty, and Y.S. Negi, Melt–solid polycondensation of lactic acid and its biodegradability. Progress in Polymer Science 34 (2009) : 99-124.

[2] Rasal, R.M., A.V. Janorkar, and D.E. Hirt, Poly(lactic acid) modifications. Progress in Polymer Science 35 (2010) : 338-356.

[3] Södergård, A. and M. Stolt, Properties of lactic acid based polymers and their correlation with composition. Progress in Polymer Science 27 (2002) : 1123-1163.

[4] Bensaid, F., et al., Y-Shaped mPEG-PLA Cabazitaxel Conjugates: Well-Controlled Synthesis by Organocatalytic Approach and Self-Assembly into Interface Drug-Loaded Core-Corona Nanoparticles. Biomacromolecules 14 (2013) : 1189-1198.

[5] Yu, Y., et al., Well-Defined Degradable Brush Polymer-Drug Conjugates for Sustained Delivery of Paclitaxel. Molecular Pharmaceutics 10 (2013) : 867-874.

[6] Cai, Q., et al., A novel porous cells scaffold made of polylactide-dextran blend by combining phase-separation and particle-leaching techniques. Biomaterials 23 (2002) : 4483-4492.

[7] Sun, H.L., et al., alpha-Amino Acid Containing Degradable Polymers as Functional Biomaterials: Rational Design, Synthetic Pathway, and Biomedical Applications. Biomacromolecules 12 (2011) : 1937-1955.

[8] Wan, Y., et al., Biodegradable polylactide/chitosan blend membranes. Biomacromolecules 7 (2006) : 1362-1372.

[9] Wan, Y.Q., et al., Biodegradable poly(L-lactide)-poly(ethylene glycol) multiblock copolymer: synthesis and evaluation of cell affinity. Biomaterials 24 (2003) : 2195-2203.

[10] Guo, B., A. Finne-Wistrand, and A.-C. Albertsson, Electroactive Hydrophilic Polylactide Surface by Covalent Modification with Tetraaniline. Macromolecules 45 (2012) : 652-659.

[11] Liu, T.Q., et al., Synthesis of polymandelide: A degradable polylactide derivative with polystyrene-like properties. Macromolecules 40 (2007) : 6040-6047.

[12] Tsuji, H., Poly(lactide) stereocomplexes: formation, structure, properties, degradation, and applications. Macromol Biosci 5 (2005) : 569-597.

[13] Garlotta, D., A literature review of poly(lactic acid). Journal of Polymers and the Environment 9 (2001) : 63-84.

[14] Mehta, R., et al., Synthesis of Poly(Lactic Acid): A Review. Journal of Macromolecular Science, Part C: Polymer Reviews 45 (2005) : 325-349.

[15] Gu, S., et al., Synthesis and characterization of biodegradable lactic acid-based polymers by chain extension. Polymer International 57 (2008) : 982-986.

[16] Liu, J.S., et al., Synthesis and chain extension of hydroxyl-terminated poly(lactic acid) oligomers and application in the blends. Polymer Composites 34 (2013) : 305-312.

[17] Liu, X., et al., Improving Melt Strength of Polylactic Acid. International Polymer Processing 28 (2013) : 64-71.

[18] Moon, S.I., et al., Melt polycondensation of L-lactic acid with Sn(II) catalysts activated by various proton acids: A direct manufacturing route to high molecular weight poly(L-lactic acid). Journal of Polymer Science Part a-Polymer Chemistry 38 (2000) : 1673-1679.

[19] Chen, G.X., et al., Synthesis of high-molecular-weight poly(L-lactic acid) through the direct condensation polymerization of L-lactic acid in bulk state. European Polymer Journal 42 (2006) : 468-472.

[20] Song, F.C. and L.B. Wu, Synthesis of High Molecular Weight Poly(L-lactic acid) via Melt/Solid Polycondensation: Intensification of Dehydration and Oligomerization During Melt Polycondensation. Journal of Applied Polymer Science 120 (2011) : 2780-2785.

[21] Hiltunen, K., et al., Synthesis and characterization of lactic acid based telechelic prepolymers. Macromolecules 29 (1996) : 8677-8682.

[22] Zeng, J.-B., et al., Synthesis and Properties of Poly(Ester Urethane)s Consisting of Poly(L-Lactic Acid) and Poly(Ethylene Succinate) Segments. Industrial & Engineering Chemistry Research 48 (2009) : 1706-1711.

[23] Inkinen, S., G.A. Nobes, and A. Sodergard, Telechelic Poly(L-lactic acid) for Dilactide Production and Prepolymer Applications. Journal of Applied Polymer Science 119 (2011) : 2602-2610.

[24] Chen, H., et al., Preparation and Characterization of poly(L-lactide) Membranes Prepared by gamma-Radiation-Induced Grafting of N-Vinyl Pyrrolidone. Journal of Applied Polymer Science 114 (2009) : 3152-3157.

[25] Kim, J.Y., et al., Synthesis of chitooligosaccharide derivative with quaternary ammonium group and its antimicrobial activity against Streptococcus mutans. International Journal of Biological Macromolecules 32 (2003) : 23-27.

[26] Fatehi, P., et al., Preparation and characterization of cationic poly vinyl alcohol with a low degree of substitution. European Polymer Journal, 47 (2011) : 997-1004.

[27] Couture, G., et al., Polymeric materials as anion-exchange membranes for alkaline fuel cells. Progress in Polymer Science 36 (2011) : 1521-1557.

[28] Baick, I.H., et al., Kinetics of Reversible Oligomerization ofl-Lactic Acid with a SnCl2·2H2O/p-Toluenesulfonic Acid Catalyst. Industrial & Engineering Chemistry Research 51 (2012) : 16617-16625.

APPENDIX

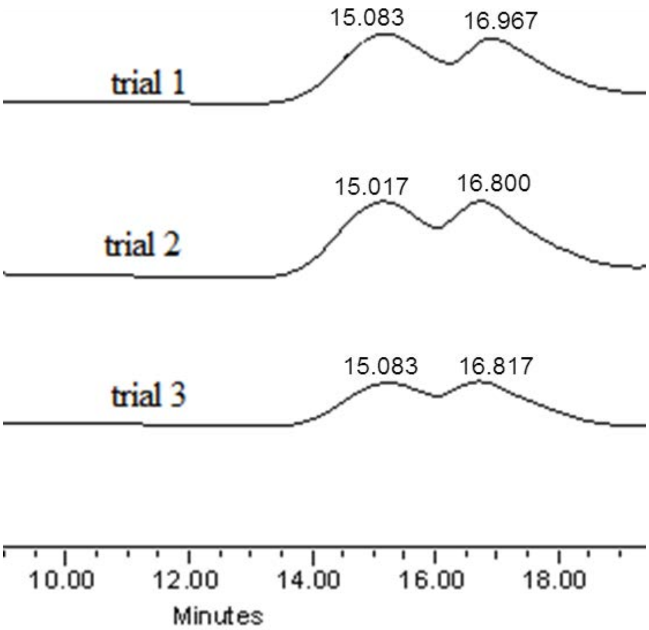


Figure A.1 GPC traces of PLLA-diCOOH+ stirred in DMF.

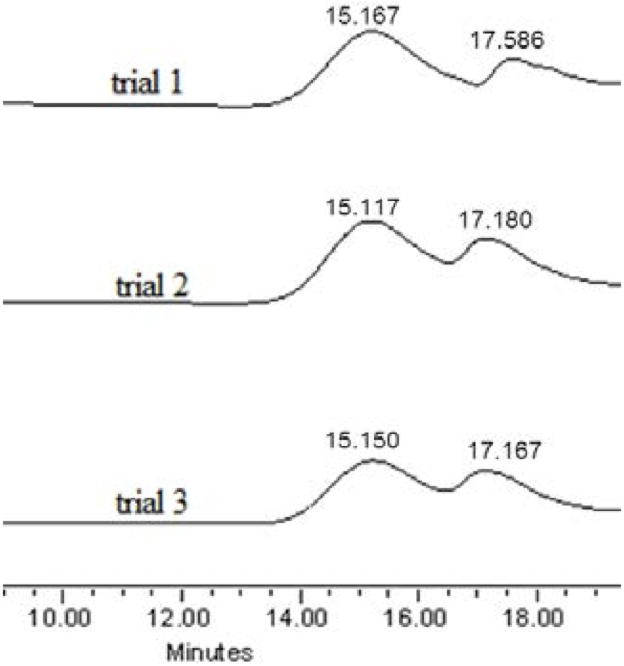


Figure A.2 GPC traces of PLLA-diCOOH+ stirred in DMF with NEt_3.

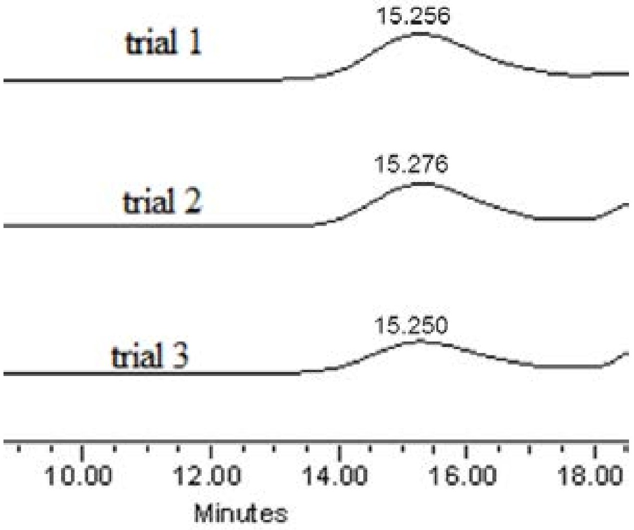


Figure A.3 GPC traces of PLLA-diCOOH+ stirred in DMSO.

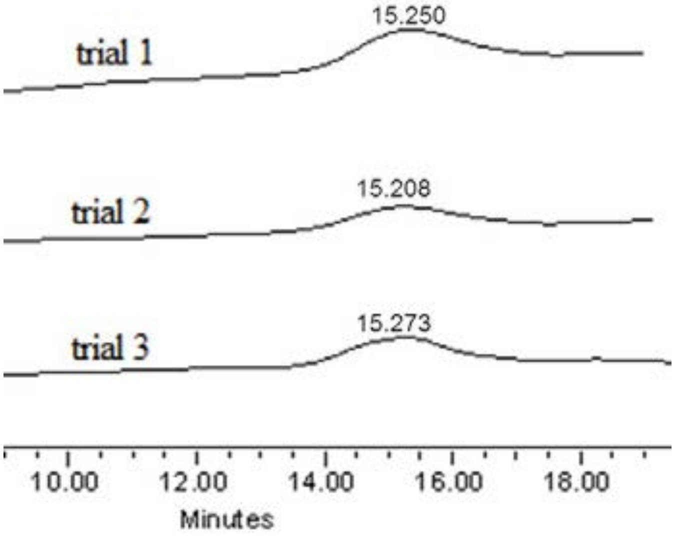


Figure A.4 GPC traces of PLLA-diCOOH+ stirred in DMSO with NEt_3.

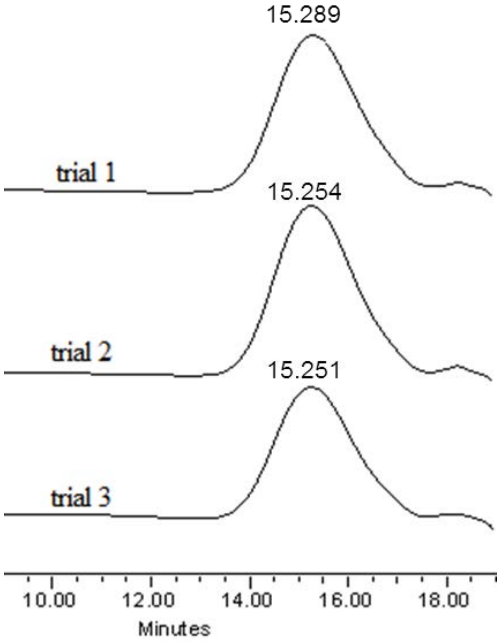


Figure A.5 GPC traces of PLLA-diOH+ stirred in DMSO.

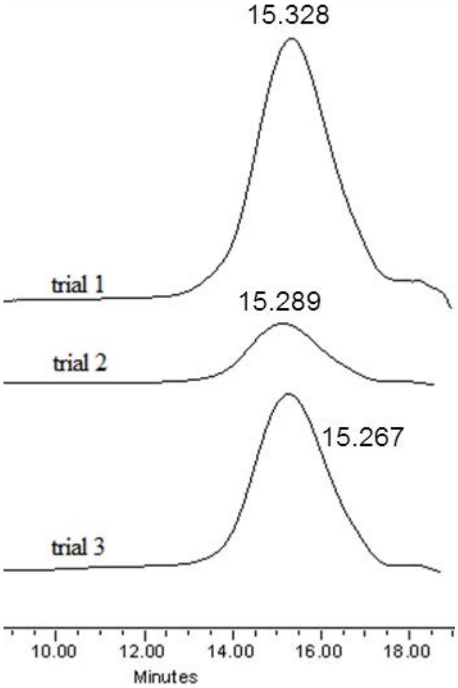


Figure A.6 GPC traces of PLLA-diOH+ stirred in DMSO with NEt_3.

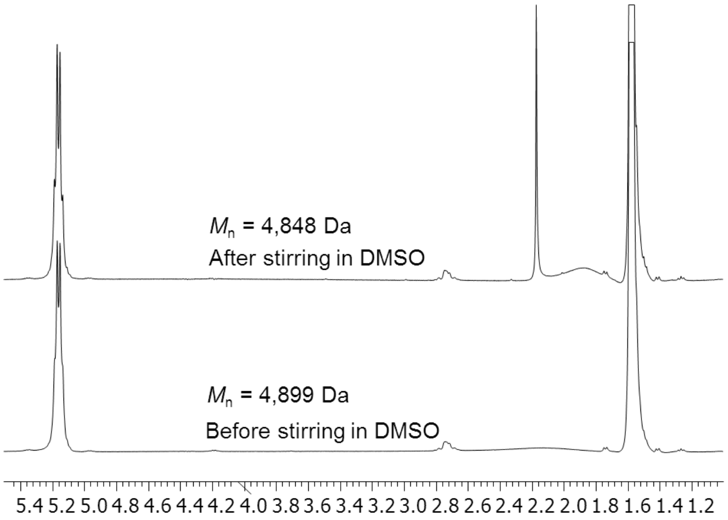


Figure A.7 ^{1}H NMR spectra of PLLA-diCOOH before and after stirring in DMSO at 70 ºC for 24 h without GTMAC.

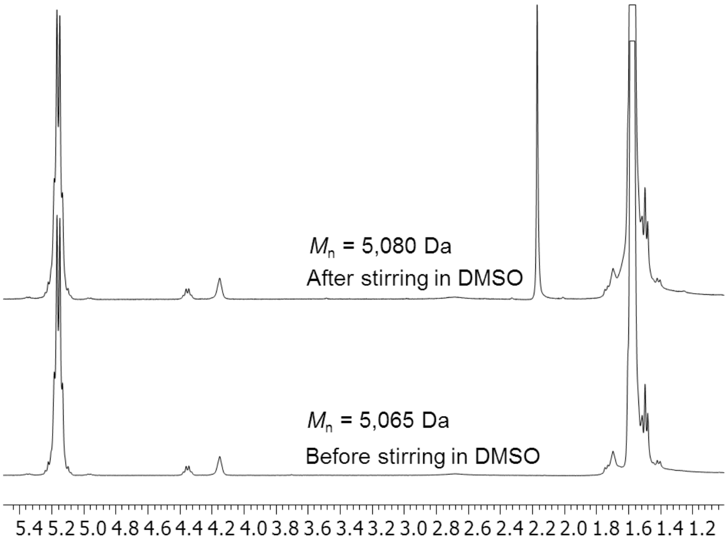


Figure A.8 ^{1}H NMR spectra of PLLA-diOH before and after stirring in DMSO at 70 ºC for 24 h without GTMAC.

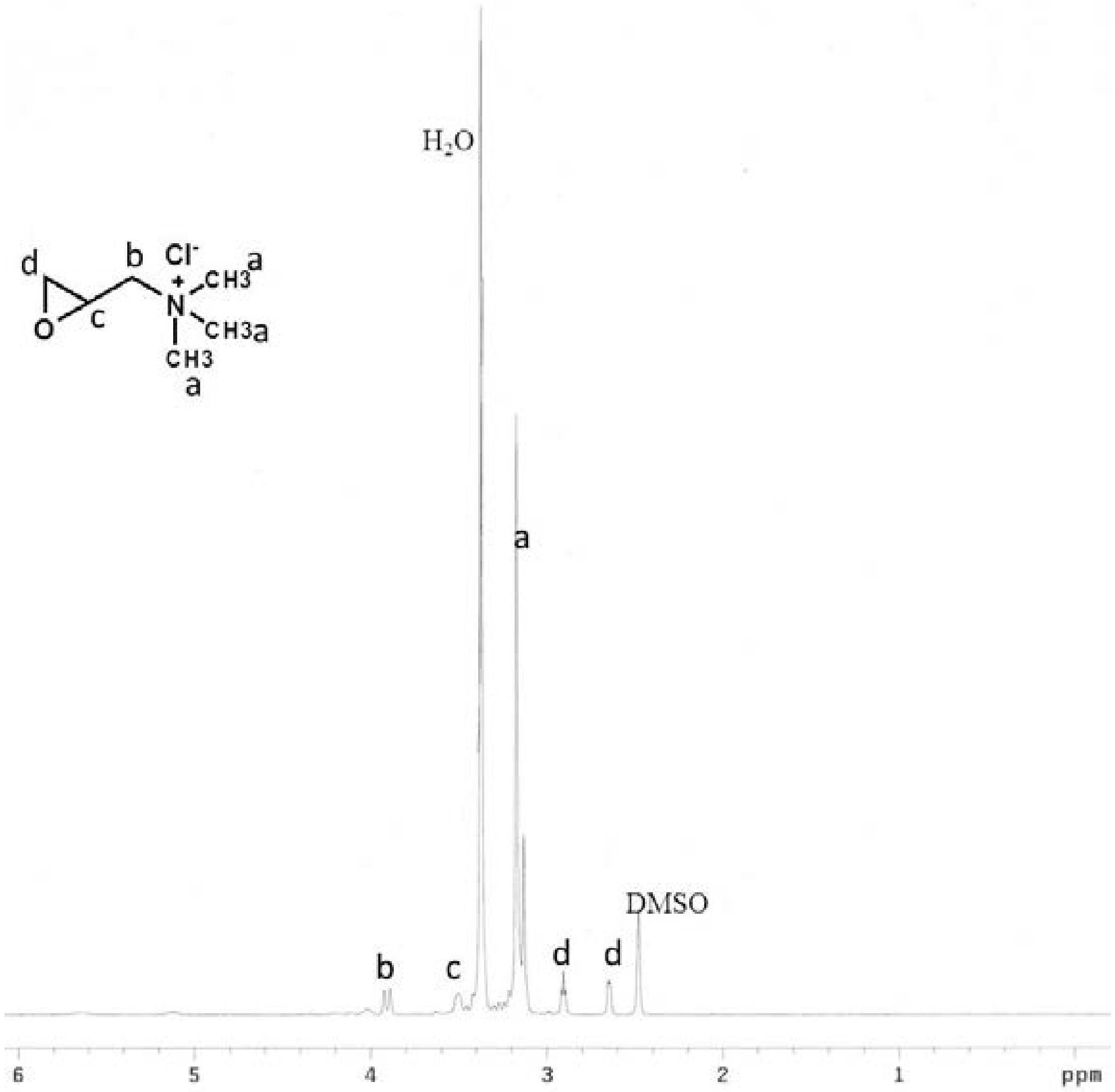


Figure A.9 ^{1}H NMR spectrum of GTMAC in (CD$_3$)SO after removing free water under high vacuum.

VITAE

Ms. Nutjarin Pansombat was born on Febuary14th, 1984 in Nakhon Phanom, Thailand. She graduated with a Bachelor's Degree of Science, majoring in Chemistry, Faculty of Science, Mahidol University in 2007. After that she worked as a chemist with 4 different companies for four years. Then she pursued her study in Master's Degree in Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University since June 2011 and finished her study in October 2013.

Presentation in Conference:

January 2013	Poster presentation in Bioplastic Conference 2013 (Inno-Bioplast 2013). Queen Sirikit National Convention Center, Bangkok, Thailand.
March 2013	Oral presentation in Polymer Conference of Thailand 2013 (PCT 2013), organized by Polymer Society of Thailand. Pathumwan Princess Hotel, Bangkok, Thailand.
October 2013	Poster presentation in the 39th Congress on Science and Technology of Thailand (STT39). BITEC, Bangkok, Thailand.